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(71) Applicant *(for all designated States except US)*: **ULTRA BIOTECH LIMITED** [GB/GB]; 2nd Floor, 60 Circular Road, Douglas, Isle of Man IM1 1SA (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **CHEUNG, Ling, Yuk** [CN/CN]; 2 Dai Fu Street,, Tai Po Industrial Estate, New Territories, Hong Kong (CN).

(74) Agents: **SETNA, Rohan, Piloo** et al.; Boult Wade Tennant, Verulam Gardens, 70 Gray's Inn Road, London WC1X 8BT (GB).

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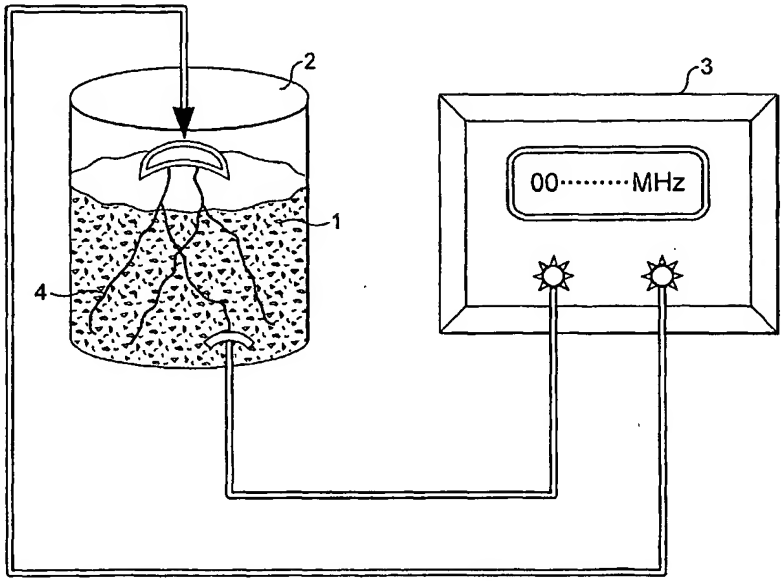
(54) Title: BIOLOGICAL COMPOSITIONS COMPRISING YEAST CELLS AND METHODS FOR TREATMENT OF CANCER



(57) Abstract: The present invention relates to biological compositions and dietary supplement comprising yeast cells that can produce a healthful benefit in a subject inflicted with cancer. The biological compositions can be used to retard the growth of cancer cells and/or prolonging the time of survival of the subject. The invention also relates to methods for manufacturing the biological compositions.

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BIOLOGICAL COMPOSITIONS COMPRISING YEAST CELLS AND METHODS FOR TREATMENT OF CANCER

RELATED APPLICATIONS

The present application claims priorities from U.S. patent applications serial nos. 10/460,246, 10/460,247, 10/460,271, 10/460,323, 10/460,324, 10/460,325, 10/460,326, 10/460,327, 10/460,328, 10/460,336, 10/460,337, 10/460,338, 10/460,341, 10/460,437, 10/460,438, 10/460,530, 10/460,832, and 10/460,833, all filed on June 11, 2003, the contents of which are herein incorporated by reference in their entireties.

FIELD OF THE INVENTION

The invention relates to biological compositions comprising yeast cells that can produce a healthful benefit in a subject inflicted with cancer. The invention also relates to methods for manufacturing the biological compositions and methods of use thereof.

BACKGROUND OF THE INVENTION

1. Cancer

Cancer is one of the leading causes of death in animals and humans. It is characterised primarily by an increase in the number of abnormal cells derived from a given normal tissue. While surgery, chemotherapeutic agents and radiation are useful in the treatment of cancer, there is a continued need to find better treatment modalities and approaches to manage the disease that are more effective and less toxic and non-invasive, especially when clinical oncologists are giving increased attention to the quality of life of cancer patients. The present invention provides an alternative approach to cancer therapy and management of the disease by using a biological composition comprising yeasts.

2. Yeast-Based Compositions

Yeasts and components thereof have been developed to be used as dietary supplement or pharmaceuticals. However, none of the prior methods uses yeast cells which have been cultured in an electromagnetic field to produce a product

that has an anti-cancer effect. The following are some examples of prior uses of yeast cells and components thereof:

United States Patent No. 6,197,295 discloses a selenium-enriched dried yeast product which can be used as dietary supplement. The yeast strain Saccharomyces boulardii sequela PY 31 (ATCC 74366) is cultured in the presence of selenium salts and contains 300 to about 6,000 ppm intracellular selenium. Methods for reducing tumor cell growth by administration of the selenium yeast product in combination with chemotherapeutic agents is also disclosed.

United States Patent No. 6,143,731 discloses a dietary additive containing whole β-glucans derived from yeast, which when administered to animals and humans, provide a source of fiber in the diet, a fecal bulking agent, a source of short chain fatty acids, reduce cholesterol and LDL, and raises HDL levels.

United States Patent No. 5,504,079 discloses a method of stimulating an immune response in a subject utilizing modified yeast glucans which have enhanced immunobiologic activity. The modified glucans are prepared from the cell wall of Saccharomyces yeasts, and can be administered in a variety of routes including, for example, the oral, intravenous, subcutaneous, topical, and intranasal route.

United States Patent No. 4,348,483 discloses a process for preparing a chromium yeast product which has a high intracellular chromium content. The process comprises allowing the yeast cells to absorb chromium under a controlled acidic pH and, thereafter inducing the yeast cells to grow by adding nutrients. The yeast cells are dried and used as a dietary supplement.

Citation of documents herein is not intended as an admission that any of the documents cited herein is pertinent prior art, or an admission that the cited documents are considered material to the patentability of the claims of the present application. All statements as to the date or representations as to the contents of these documents are based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these documents.

SUMMARY OF THE INVENTION

The present invention relates to biological compositions useful for treatment of subjects with cancer. In one embodiment, the present invention provides

biological compositions comprising live yeast cells which are capable of producing a healthful benefit in subjects with cancer. In other embodiments, the invention provides methods of making the biological compositions, and methods of using the biological compositions.

In particular, the methods of the invention comprise culturing yeast cells in the presence of a series of electromagnetic fields such that the yeast cells becomes metabolically active. The electromagnetic fields used are each defined by one of five frequency ranges and a broad range of field strength. The starting yeast cells are commercially available and/or accessible to the public, such as but not limited to *Saccharomyces*. The methods for making the biological compositions of the invention may further comprise conditioning the activated yeast cells in plant extracts and the gastric juice of animals, while in the presence of another series of electromagnetic fields.

The methods of manufacturing also comprise expanding the number of activated or activated and conditioned yeast cells in large scale cultures in the presence of yet another series of electromagnetic fields, performing quality control measures, and packaging. Pharmaceutical compositions of the invention comprises activated and conditioned yeast cells and one or more pharmaceutically acceptable excipients or carriers. Additional ingredients, such as vitamins, herbs, and/or flavors may be added to the biological compositions to form the oral compositions of the invention. Such additional carriers and ingredients can improve the healthful benefits, pharmacological properties, and organoleptic characteristics of the oral compositions. During the manufacturing process, the activated or activated and conditioned yeast cells may be dried and stored for a period of time.

The biological or oral compositions of the invention are ingested by the subject or used as an additive to be incorporated into food to be consumed by the subject. Dietary supplement and nutritional compositions comprising activated and conditioned yeast cells are encompassed by the invention. Preferably, the subject is a human being.

In various embodiments, the biological compositions of the invention are used to produce a healthful benefit in a subject with cancer or at high risk of developing cancer. In particular, the biological composition of the invention can retard the growth of cancer cells in an animal which received the composition orally.

The biological composition can also be used to prolong the time of survival of an animal with cancer.

BRIEF DESCRIPTION OF FIGURES

Fig. 1 Activation and conditioning of yeast cells. 1 yeast cell culture; 2 container; 3 electromagnetic field source; 4 electrode.

Fig. 2 Large scale propagation of yeast cells. 5 first container; 6 second container; 7 third container; 8 yeast cell cultures; 9 electromagnetic field source.

DETAILED DESCRIPTION OF THE INVENTION

1. General

The present invention relates to biological compositions that can produce a healthful benefit in a subject with cancer. The present invention provides methods for manufacturing the biological compositions as well as methods for using the biological compositions.

The term "cancer" as used herein refers to all types of cancers, or neoplasms or benign or malignant tumors, including but not limited to carcinoma, sarcoma, lymphoma, and leukemia. In particular, the term "cancer" as used herein includes but is not limited to lung cancer, nasopharyngeal cancer, esophageal cancer, stomach cancer, colorectal cancer, pancreatic cancer, liver cancer, testicular cancer, prostate cancer, ovarian cancer, breast cancer, cervical cancer, uterine cancer, kidney cancer, bladder cancer, brain cancer, lymphoma, and leukemia.

In one embodiment, the invention provides biological compositions that comprise yeasts. Unlike the traditional use of yeasts in the making of food, the yeast cells of the invention are not used as a source of enzymes that acts on the food ingredients. The yeasts are not a primary source of nutrients for the subject. Nor are yeast cells used as a carrier of an active ingredient, such as metal salts. The yeast cells of the invention are live when administered orally or ingested along with food by a subject. Without being bound by any theory or mechanism, the inventor believes that the culture conditions activate and/or amplified the expression of a gene or a set of genes in the yeast cells such that the yeast cells become highly effective in stimulating the animal's immune system, including both specific and non-specific

immunological reactions, the results of which are manifested as the overall healthful benefits observed in the treated subject. The healthful benefits provided by using the biological compositions are demonstrated in animal models of human cancers, which show inhibition of tumor growth and prolonged survival time of animals with the disease.

In another embodiment, the invention provides methods for making the yeast cells in the biological compositions. The starting materials are normal yeast cells which can be readily obtained commercially or from public microorganism deposits. The methods of the invention comprise a set of culture conditions that can be applied reproducibly to activate the yeast cells. The key feature of the culture conditions used in the methods of the invention is a series of alternating electromagnetic fields of defined frequency ranges and field strengths which are applied to the growing yeast cell culture. The method further comprises the step of conditioning the activated live yeast cells to the acidic environment of the stomach of the subject. The electromagnetic fields used in these methods can be created reproducibly at various scales, thus enabling even the large scale manufacturing of the biological compositions of the invention. By careful control of the culturing conditions, normal yeast cells can be activated routinely and reproducibly to become yeast cells of the invention.

In yet another embodiment, the invention provides methods for manufacturing a biological composition comprising activated and conditioned yeasts of the invention, and additional ingredients, including but not limited to pharmaceutically acceptable carriers or excipients, vitamins, herbs (including traditional Chinese medicine products), herbal extracts, minerals, amino acids, flavoring agents, coloring agents, and/or preservatives.

In yet another embodiment, the biological compositions can be added to food which will be consumed by the subject. As known to those skilled in the relevant art, many methods may be used to mix the biological or oral compositions of the invention with food while the yeast cells remain viable. In a particular embodiment, the culture broth comprising live yeast cells of the present invention are added directly to food just prior to consumption. Dried powders of the yeasts can also be reconstituted and added directly to food just prior to consumption.

In various embodiments, the oral compositions of the invention can be consumed directly by a subject or be fed directly to a subject. For example, the

subject may drink the culture broth or a fraction thereof that comprises live activated and conditioned yeast cells. Oral compositions comprising dried yeast cells can also be given as a solid dosage form to the subject.

Although it is not necessary, the biological or oral compositions of the invention can be used in conjunction or in rotation with other types of treatment modalities such as but not limited to surgery, chemotherapeutic agents, and radiation. Since the biological compositions of the invention are administered orally, the assistance of health professionals in administration of the composition is generally not essential.

Described below are the yeast cells of the invention and methods of their preparation, followed by descriptions of using the biological compositions of the invention in a subject suffering from cancer. The examples of using the invention in treatment of eighteen (18) commonly occurring cancers are also provided to further demonstrate the therapeutic benefits of an oral composition of the invention. The activated and conditioned yeast cells in the oral composition are characterized by their ability to (i) suppress the growth of cancer cells in an animal model of human cancer or (ii) prolong the survival of animals with transplanted cancer cells in a model of human cancer as compared to yeast cells which have not been activated and conditioned.

2. Yeast Cells

The yeast cells of the biological composition are produced by culturing a plurality of yeast cells in an appropriate culture medium in the presence of an alternating electromagnetic field over a period of time. The method comprises a first step of activating the yeast cells and a second step of conditioning the activated yeast cells. The activation process comprises culturing yeast cells in the presence of at least two, three, four or five electromagnetic fields of specific frequencies and field strength. The conditioning process comprises further culturing of the activated yeast cells in a medium comprising plant extracts and extracts from the stomach of an animal, in the presence of at least one electromagnetic field. The activated and conditioned yeast cells can be stored as dried cells after drying the cells under appropriate conditions. The dried activated and conditioned yeast cells can be used later in large scale culturing processes for manufacturing the biological compositions

of the invention. The various culturing processes of the invention can be performed either as a batch process or a continuous process.

In various embodiments, yeasts of the genera of Saccharomyces, Candida, Crebrothecium, Geotrichum, Hansenula, Kloeckera, Lipomyces, Pichia, Rhodosporidium, Rhodotorula, Torulopsis, Trichosporon, and Wickerhamia can be used in the invention. Generally, fungi used for food manufacturing are preferred.

Non-limiting examples of yeast strains include *Saccharomyces sp.*, AS2.311; *Schizosaccharomyces pombe* Linder, AS2.214, AS2.248, AS2.249, AS2.255, AS2.257, AS2.259, AS2.260, AS2.274, AS2.994, AS2.1043, AS2.1149, AS2.1178, IFFI 1056; *Saccharomyces sake* Yabe, ACCC2045; *Saccharomyces uvarum* Beijer, IFFI 1023, IFFI 1032, IFFI 1036, IFFI 1044, IFFI 1072, IFFI 1205, IFFI 1207; *Saccharomyces rouxii* Boutroux, AS2.178, AS2.180, AS2.370, AS2.371; *Saccharomyces cerevisiae* Hansen Var. ellipsoideus, ACCC2043, AS2.2, AS2.3, AS2.8, AS2.53, AS2.163, AS2.168, AS2.483, AS2.541, AS2.559, AS2.606, AS2.607, AS2.611, AS2.612; *Saccharomyces carlsbergensis* Hansen, AS2.116, AS2.162, AS2.189, AS2.200, AS2.216, AS2.265, AS2.377, AS2.417, AS2.420, AS2.440, AS2.441, AS2.443, AS2.444, AS2.459, AS2.595, AS2.605, AS2.638, AS2.742, AS2.745, AS2.748, AS2.1042; *Rhodotorula aurantiaca* (Saito)Ladder; AS2.102, AS2.107, AS2.278, AS2.499, AS2.694, AS2.703, AS2.704 and AS2.1146; *Saccharomyces cerevisiae* Hansen, ACCC2034, ACCC2035, ACCC2036, ACCC2037, ACCC2038, ACCC2039, ACCC2040, ACCC2041, ACCC2042, AS2.1, AS2.4, AS2.11, AS2.14, AS2.16, AS2.56, AS2.69, AS2.70, AS2.93, AS2.98, AS2.101, AS2.109, AS2.110, AS2.112, AS2.139, AS2.173, AS2.182, AS2.196, AS2.242, AS2.336, AS2.346, AS2.369, AS2.374, AS2.375, AS2.379, AS2.380, AS2.382, AS2.393, AS2.395, AS2.396, AS2.397, AS2.398, AS2.399, AS2.400, AS2.406, AS2.408, AS2.409, AS2.413, AS2.414, AS2.415, AS2.416, AS2.422, AS2.423, AS2.430, AS2.431, AS2.432, AS2.451, AS2.452, AS2.453, AS2.458, AS2.460, AS2.463, AS2.467, AS2.486, AS2.501, AS2.502, AS2.503, AS2.504, AS2.516, AS2.535, AS2.536, AS2.558, AS2.560, AS2.561, AS2.562, AS2.576, AS2.593, AS2.594, AS2.614, AS2.620, AS2.628, AS2.631, AS2.666, AS2.982, AS2.1190, AS2.1364, AS2.1396, IFFI 1001, IFFI 1002, IFFI 1005, IFFI 1006, IFFI 1008, IFFI 1009, IFFI 1010, IFFI 1012, IFFI 1021, IFFI 1027, IFFI 1037, IFFI 1042, IFFI 1045, IFFI 1048, IFFI 1049, IFFI 1050, IFFI 1052, IFFI 1059, IFFI 1060, IFFI 1062, IFFI 1202, IFFI 1203, IFFI 1209, IFFI 1210, IFFI 1211, IFFI 1212, IFFI 1213,

IFFI 1215, IFFI 1221, IFFI 1224, IFFI 1247, IFFI 1248, IFFI 1251, IFFI 1270, IFFI 1277, IFFI 1289, IFFI 1290, IFFI 1291, IFFI 1292, IFFI 1293, IFFI 1297, IFFI 1300, IFFI 1301, IFFI 1302, IFFI 1307, IFFI 1308, IFFI 1309, IFFI 1310, IFFI 1311, IFFI 1331, IFFI 1335, IFFI 1336, IFFI 1337, IFFI 1338, IFFI 1339, IFFI 1340, IFFI 1345, IFFI 1348, IFFI 1396, IFFI 1397, IFFI 1399, IFFI 1441 and IFFI 1443. Preferred yeast strains include but are not limited to *S. cerevisiae* AS2.501, AS2.502, AS2.503, AS2.504, AS2.535, AS2.558, AS2.560, AS2.561 and AS2.562.

Generally, yeast strains useful for the invention can be obtained from private or public laboratory cultures, or publicly accessible culture deposits, such as the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 and the China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture Collection of Microorganisms, Institute of Microbiology, Chinese Academy of Sciences, Haidian, P.O. Box 2714, Beijing, 100080, China.

Non-limiting examples of using yeast cells of the invention are provided below. The yeast cells of the invention do not comprise an enhanced level of selenium or chromium relative to that found in naturally occurring yeast cells. In certain embodiments, the biological compositions do not comprise cells of *Saccharomyces boulardii* (for example, ATCC Accession No. 74366) or cells of a particular strain of *Saccharomyces cerevisiae* (strain Hansen CBS 5926) that is also commonly referred to as *Saccharomyces boulardii.*

Although it is preferred, the preparation of the yeast cells of the invention is not limited to starting with a pure strain of yeast. The yeast cells in the biological compositions may be produced by culturing a mixture of yeast cells of different species or strains. The constituents of a mixture of yeast cells can be determined by standard yeast identification techniques well known in the art.

In various embodiments of the invention, standard techniques for handling, transferring and storing yeasts are used. Although it is not necessary, sterile conditions or clean environments are highly desirable when carrying out the manufacturing processes of the invention, especially when the biological compositions are for human consumption. The manufacturing process can be adapted to meet regulatory guidelines on product safety and quality control by standard practice known in the art.

3. Electromagnetic Fields

As used herein, the terms "alternating electromagnetic field", "electromagnetic field" or "EM field" are synonymous. An electromagnetic field useful in the invention can be generated by various means well known in the art. A schematic illustration of exemplary setups are depicted respectively in Figure 1. An electromagnetic field of a desired frequency and a desired field strength is generated by an electromagnetic wave source (3) which comprises one or more signal generators that are capable of generating electromagnetic waves, preferably sinusoidal waves, and preferably in the frequency range of 1,500 to 15,000 MHz and most preferably 7,800 to 12,900 MHz. Such signal generators are well known in the art. Signal generators capable of generating signal with a narrower frequency range can also be used. If desirable, a signal amplifier can also be used to increase the output signal, and thus the strength of the EM field.

The electromagnetic field can be applied to the culture by a variety of means including placing the yeast cells in close proximity to a signal emitter connected to a source of electromagnetic waves. The signal generator is connected to the signal emitter by cables such as coaxial cables that can transmit signals up to greater than or equal to 30 GHz. Typically, the yeast cells are placed in a container which is made of material that is not an electric conductor, such as but not limited to plastic, resin, glass, and ceramic.

In one embodiment, the electromagnetic field is applied by signal emitters in the form of electrodes (4) that are submerged in a culture of yeast cells (1). In a preferred embodiment, one of the electrodes is a metal plate which is placed on the bottom of a non-conducting container (2), and the other electrode comprises a plurality of wires or tubes so configured inside the container such that the energy of the electromagnetic field can be evenly distributed in the culture. The electrodes are preferably made of copper. For an upright culture vessel, the tips of the wires or tubes are placed within 3 to 30 cm from the bottom of the vessel (i.e., approximately 2% to 10% of the height of the vessel from the bottom). Table 1 provides exemplary set up for culturing the yeast cells of the invention.

Table 1

Height of culture medium in the non-conducting	Distance electrodes are placed from the bottom	Range for distance of the electrodes from the

container (cm)	of the container (cm)	bottom (cm)
15 to 20	3	3 to 5
20 to 30	5	5 to 7
30 to 50	7	7 to 10
50 to 70	10	10 to 15
70 to 100	15	15 to 20
100 to 150	20	20 to 30
150 to 200	30	25 to 30

The number of electrodes used depends on both the volume of the culture and the diameter of the electrode. For example, for a culture having a volume of 10 liter or less, two or three electrodes having a diameter of between 0.5 to 2.0 mm can be used. For a culture volume of 10 to 100 liter of culture, the electrodes can have a diameter of 3.0 to 5.0 mm. For a culture volume of 100 to 1,000 liter, the electrodes can have a diameter of 6.0 to 15.0 mm. For a culture having a volume greater than 1,000 liter, the electrodes can have a diameter of between 20.0 to 25.0 mm.

4. Activation of Yeast Cells

According to the invention, the method for producing activated yeast cells of the invention comprises culturing yeast cells in the presence of at least two, three, four or five alternating electromagnetic (EM) fields.

The culture process can be initiated by inoculating 1,000 ml of medium with an inoculum of a selected yeast strain (such as one of those described above) such that the starting cell density of the culture is greater than about 10^5 cells per ml. The starting culture can be used to seed larger scale culture. The culture is maintained initially at 28°C to 32°C for 22 to 30 hours prior to exposure to the EM field(s), typically at 30°C for 28 hours.

The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.08 mol/m^3, preferably 0.04 mol/m^3. The oxygen level can be controlled by any conventional means known in the art, including but not limited to stirring and/or bubbling.

The culture is most preferably carried out in a liquid medium which contains sources of nutrients assimilable by the yeast cells, such as sucrose or glucose, vitamin H, vitamin B_6, vitamin B_{12}, fetal calf serum, peptone, and inorganic ions such as K^+, Na^+, Mg^{2+}, Ca^{2+}, Cl^-, CO_3^{2-}, PO_4^{3-}, and SO_4^{2-}. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and is preferably between about 0.2% and 2%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium. Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are KH_2PO_4, $(NH_4)_2HPO_4$, $CaCO_3$, $MgSO_4$, NaCl, and $CaSO_4$.

In one embodiment, a medium containing one or more of sucrose or glucose (10-30 g), soluble starch (10 g), mannitol (10 g), vitamin A (40-60 μg), vitamin B_2 (40-50 μg), vitamin B_3 (40-60 μg), vitamin B_6 (30-80 μg), vitamin B_{12} (30-60 μg), vitamin C (40-50 μg), vitamin D (20 μg), vitamin H (20-70 μg), bovine calf serum (30-35 ml), fetal calf serum (25-45 ml), KH_2PO_4 (0.20 g), $MgSO_4.7H_2O$ (0.20-0.25 g), NaCl (0.20-0.30 g), $CaSO_4.2H_2O$ (0.20-0.30 g), $CaCO_3.5H_2O$ (3.0-4.0 g), peptone (2.5 g), and autoclaved water (up to 1,000 ml) can be used to culture the yeast cells of the invention. However, media containing ingredients other than the ones listed above can also be used in culturing the same or different strains of yeast cells. Preferably, the culturing medium is heated to 45°C and cooled before adding nutrients such as vitamin A, vitamin B_2, vitamin B_3, vitamin B_6, vitamin B_{12}, vitamin C, vitamin D, vitamin H, bovine calf serum and/or fetal calf serum.

It should be noted that the composition of the media provided herein is not intended to be limiting. The process can be scaled up or down according to needs. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

In certain embodiments, a series of at least two, three, four or five EM fields are applied to the culture of yeast cells, each having a different frequency

within a stated range, and a different field strength within a stated range. The EM fields can be applied in any order and by any means known in the art, such as the apparatus described above. Although any of the following two, three or four EM fields can be applied, preferably, all five EM fields are applied.

For the first EM field, the frequency is in the range of 7,821 to 10,170 MHz and the field strength is in the range of 200 to 350 mV/cm. The yeast culture is exposed to this first EM field at 30 ± 2°C for about 5 to 36 hours.

For the second EM field, the frequency is in the range of 7,993 to 11,530 MHz and the field strength is in the range of 190 to 330 mV/cm. The yeast culture is exposed to this second EM field at 30 ± 2°C for about 4 to 36 hours.

For the third EM field, the frequency is in the range of 9,907 to 12,285 MHz and the field strength is in the range of 230 to 430 mV/cm. The yeast culture is exposed to this third EM field at 30 ± 2°C for about 10 to 34 hours.

For the fourth EM field, the frequency is in the range of 11,141 to 12,842 MHz and the field strength is in the range of 220 to 450 mV/cm. The yeast culture is exposed to this fourth EM field at 30 ± 2°C for about 4 to 34 hours.

For the fifth EM field, the frequency is in the range of 12,031 to 12,900 MHz and the field strength is in the range of 260 to 450 mV/cm. The yeast culture is exposed to this fifth EM field at 30 ± 2°C for about 5 to 34 hours.

In less preferred embodiments, the yeast cells can be cultured by exposure to two, three or four of the above-mentioned EM fields in a different order. The yeast cells can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The cell density of the culture at the end of the activation process is typically greater than about 10^6 to 10^9 cells per ml (estimated by hematocytometer). The activated yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C to 4°C. The activated yeast cells recovered from the liquid culture may be dried and stored in powder form. Preferably, the powder form of the yeast cells comprises greater than about 10^7 to 10^{10} yeast cells per gram.

5. Conditioning of Yeast Cells

According to the invention, performance of the activated yeast cells can be optimized by culturing the activated yeast cells in the presence of an extract from the stomach (e.g., the gastric juice) of an animal with physiology similar to the subject to which the biological composition will be administered. The inclusion of this additional conditioning process allows the activated yeast cells to adapt to and endure the acidic environment of the subject's stomach. The method for conditioning activated yeast cells of the invention comprises culturing yeast cells in such materials in the presence of at least one EM field.

The culture process can be initiated by inoculating 1,000 ml of a conditioning medium with about 10 gram of dried activated yeasts containing about 10^{10} cells per gram (as prepared by the methods described above). An equivalent number of yeast cells in culture, preferably greater than 10^6 to 10^9 cells per ml, more preferably at 10^8 cells per ml, can also be used as an inoculum. The conditioning medium comprises per 1,000 ml about 700 ml of gastric juice of an animal and about 300 ml of wild hawthorn juice. The process can be scaled up or down according to needs.

The gastric juice of an animal can be obtained from the stomach content of a freshly slaughtered animal. Although not essential, the animal is preferably kept under a clean environment, and fed a standard diet, preferably germ-free. For example, the content of the stomach of a 120-day old pig is mixed with 2,000 ml of distilled water, and allowed to settle without stirring for 6 hours. The clear liquid above is collected for use as the gastric juice used in the conditioning process. The gastric juice of a pig can be used to condition yeast cells for use in a variety of mammals, including humans. Other methods that can be used to collect the gastric juice include centrifugation or filtration of the mixture to remove debris and/or microorganisms. The gastric juice so obtained can be stored at 4°C. Preferably, the collection procedures and storage are carried out under sterile conditions.

The wild hawthorn juice is an extract of wild hawthorn fruits prepared by slicing the fruits and drying the slices in air, preferably to less than 8% moisture (commercial dryer can be used if necessary), crushing the dried fruits to less than 20 mesh, and mixing 1,500 ml of water per 500 gram of the crushed wild hawthorn. The mixture is then allowed to settle without stirring for 6 hours, and the clear liquid above is collected for use as the wild hawthorn juice used in the conditioning process. Other methods that can be used to collect the hawthorn juice include centrifugation or

filtration of the mixture. Preferably, the collection procedures and storage are carried out under sterile conditions.

The activated yeast cells are conditioned by culturing in at least one of the following two EM fields which can be applied by the apparatus described above or any means known in the art:

The first EM field has a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 230 to 440 mV/cm. The temperature is maintained at 28°C to 32°C, and typically at 30°C. The yeast culture is exposed to this first EM field for about 4 to 50 hours.

The second EM field has a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm. The temperature is maintained at 28°C to 32°C, and typically at 30°C. The yeast culture is exposed to this second EM field for 10 to 52 hours.

In a preferred embodiment, the activated yeast cells are conditioned by culturing in both of the above-mentioned EM fields. In less preferred embodiments, the yeast cells are conditioned in the two different EM fields in a different order. In other embodiments, a series of EM fields having field characteristics within the ranges stated above can be applied to condition the yeast cells. The yeast cells can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The cell density of the culture at the end of the activation and conditioning process is typically greater than about 10^7 to 10^{10} cells per ml (estimated by hematocytometer). The activated and conditioned yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C to 4°C.

The activated and conditioned yeast cells can be used directly in a biological composition or used as a starter culture for large scale manufacturing. The activated and conditioned yeast cells recovered from the liquid culture may be dried and stored in powder form. Preferably, the powder form of the activated and conditioned yeast cells comprises greater than about 10^8 to 10^{11} yeast cells per gram.

6. Large Scale Manufacturing

The present invention also encompasses methods of manufacturing of the biological compositions of the invention at a large scale. The activated and

conditioned yeast cells as prepared above are propagated on a large scale to make the biological compositions of the invention. The method comprises culturing the yeast cells in the presence of one or more EM fields for a period of time, diluting the growing yeast cells with fresh medium, and repeating the process. The method can be carried out as a batch process or a continuous process.

In one preferred embodiment, a set of three containers (5, 6, 7) each comprising a set of electrodes for generating an electromagnetic field as described above are set up each with 1,000 liters of a culture medium. See Figure 2. The culture medium comprises nutrients assimilable by the yeast cells as shown in Table 2.

Table 2

Material	**Quantity**
Wild hawthorn juice	300 liters
Jujube juice	300 liters
Wu wei zi juice	300 liters
Soybean juice	100 liters

The wild hawthorn juice is an extract of fresh wild hawthorn fruits prepared by washing the fruits clean, drying the fruits in air or using a commercial dryer to less than 8% moisture, crushing the dried fruits to less than 20 mesh, and mixing the crushed wild hawthorn with water at a ratio of 400 liters of water per 100 kg of crushed fruits. The mixture is then stirred continuously for 12 hours while the temperature is maintained at 28°C to 30°C. The mixture is then centrifuged at 1,000 rpm to collect the supernatant which is used as described above. Preferably, the procedures are carried out under sterile conditions.

The jujube juice is an extract of fresh jujube fruits prepared by washing the fruits clean, drying the fruits to less than 8% moisture, crushing the dried fruits to less than 20 mesh, and mixing the crushed jujube with water at a ratio of 400 liters of water per 100 kg of crushed fruits. The mixture is then stirred continuously for 12 hours while the temperature is maintained at 28°C to 30°C. The mixture is then

centrifuged at 1,000 rpm to collect the supernatant which is used as described above. Preferably, the procedures are carried out under sterile conditions.

The wu wei zi juice is an extract of fresh berries of *Schisandra chinensis* plant prepared by washing the berries, drying the fruits to less than 8% moisture, crushing the dried berries to less than 20 mesh, and mixing the crushed berries with water at a ratio of 400 liters of water per 100 kg of crushed berries. The mixture is then stirred continuously for 12 hours while the temperature is maintained at 28°C to 30°C. The mixture is then centrifuged at 1,000 rpm to collect the supernatant which is used as described above. Preferably, the procedures are carried out under sterile conditions.

The soybean juice is prepared by washing the soybeans, drying the soybeans to less than 8% moisture, crushing the soybeans to less than 20 mesh, and mixing the crushed soybeans with water. For 30 kg of soybeans, 130 liters of water is used. The mixture is then stirred continuously for 12 hours while the temperature is maintained at 28°C to 30°C. The mixture is then centrifuged at 1,000 rpm to collect the supernatant which is used as described above. Preferably, the procedures are carried out under sterile conditions.

The first container is inoculated with activated or activated and conditioned yeast cells as prepared by the methods as set forth above. About 1,000 gram of dried yeast powder are added to 1,000 liter of culture medium. Each gram of the dried yeast powder comprises about 10^{10} yeast cells. Instead of dried yeast cells, an equivalent number of yeast cells in a liquid medium can also be used, preferably greater than about 10^6 to 10^9 cells per ml, more preferably about 10^7 cells per ml.

The yeast cells in the first container (5) are then subjected to a series of two EM fields. For the first EM field, which can be applied by the apparatus described above, the frequency is in the range of 11,141 to 12,842 MHz and the field strength is in the range of 170 to 450 mV/cm. The yeast culture is exposed to this first EM field for about 4 to 25 hours. The yeast cells are then subjected to a second EM field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 120 to 450 mV/cm. The yeast culture is exposed to this second EM field for about 4 to 24 hours. The yeast cells from the first container are then transferred to the second container which contains about 1,000 liter of the culture medium. In effect, the first yeast culture is diluted by about 50% with fresh culture medium.

In the second container (6), the yeast cells are again subjected to a series of two EM fields. The frequencies used in the second container are similar to those used in the first container. The yeast cells from the second container are then transferred to the third container which contains yet another 1,000 liter of the culture medium. Again, the second yeast culture is diluted by about 50% with fresh culture medium.

In the third container (7), the yeast cells are again subjected to a series of two EM fields. The frequencies used in the third container are similar to those used in the first and second container.

The yeast cell culture resulting from the end of this stage can be used directly as an oral composition of the invention, or used to form other compositions encompassed by the invention.

The cell density of the culture at the end of the large scale manufacturing process is typically greater than about 10^8 to 10^{10} cells per ml (estimated by hematocytometer). The concentration of yeast cells in the medium can be concentrated or diluted accordingly. In certain embodiments, the concentration of yeast cells in the medium is in the range of 10^3 to 10^{10} cells per ml. In less preferred embodiments, the concentration of yeast cells in the medium is in the range of 10^3 to 10^6 cells per ml. In more preferred embodiments, the concentration of yeast cells in the medium is greater than 10^6 to 10^{10} cells per ml. In most preferred embodiments, the concentration of yeast cells in the medium is in the range of 10^6 to 5×10^8 cells per ml.

Other ingredients that enhance the healthful benefits, pharmacological properties and/or organoleptic characteristics of the composition can be added to the yeast cell culture. To maintain viability and freshness of the composition, it is preferred that the various downstream and packaging process be carried out below room temperature, and preferably at 0°C to 4°C. In one embodiment, the yeast cell culture can be packaged in liquid containers.

In another embodiment, the activated and conditioned yeast cells can be dried as follows. The yeast cell culture is first centrifuged under 75 to 100 g for 10 to 20 minutes to remove the supernatant. The residue which may contain up to 85% moisture is dried in a first dryer at a temperature not exceeding 60 ± 2°C for a period of 5 minutes so that yeast cells quickly became dormant. The yeast cells were then sent to a second dryer and dried at a temperature not exceeding 65 ± 2°C for a period

of about 8 minutes to further remove at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% of water. For example, the yeast cells may be dried to remove at least 88% of water so the dried yeast cells may contain up to 12% moisture.

After cooling to room temperature, the dried yeast cells can be packaged by standard pharmaceutical methods in various solid dosage form, each containing a predetermined amount of the dried material. In a preferred embodiment, the dried material comprises about 10^5 to 10^{11} cells per gram. In a more preferred embodiment, the dried material comprises about 10^8 to 5×10^{10} cells per gram. In a most preferred embodiment, the dried material comprises about 5×10^8 cells per gram.

In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers.

7. Methods of Use

The present invention further provides methods of use of the biological compositions of the invention. In one embodiment, the biological composition is used as a medicament for treatment of cancer. In another embodiment, the biological composition is used as a dietary supplement, health food, or health drink. The methods comprise administering an effective amount of the biological composition to a subject in need. The biological composition may be administered orally, in liquid or solid form, or enterally through a feeding tube. As used herein, the term "an effective amount" means an amount sufficient to provide a therapeutic or healthful benefit in the context of cancer.

According to the invention, the biological composition can produce a healthful benefit in a subject suffering from cancer. The subject is preferably a mammal such as a non-primate (*e.g.*, a cow, pig, horse, cat, dog, rat, mouse, rabbit, etc.) or a primate (*e.g.*, a monkey, chimpanzee, human, etc.). In one embodiment, the subject is non-human. Most preferably, the subject is a human being.

In one embodiment, the subject in need is one who is diagnosed with cancer with or without metastasis, at any stage of the disease (e.g., TNM staging by the American Joint Committee for Cancer (AJCC) published in 1988, or other staging system acceptable in the art). The subject may be a cancer patient who is receiving concurrently other treatment modalities against the cancer. The subject can be a

cancer patient who had undergone a regimen of treatment (e.g., chemotherapy and/or radiation) and whose cancer is regressing. The subject may be a cancer patient who had undergone a regimen of treatment (e.g., surgery) and who appears to be clinically free of the cancer. The biological composition of the invention can be administered adjunctively with any of the treatment modalities, such as but not limited to chemotherapy, radiation, and/or surgery. For example, the biological composition can be used in combination with one or more chemotherapeutic or immunotherapeutic agents, such as anastrozole (Arimidex®), amsacrine (AMSA), L-asparaginase (Elspar®), bleomycin, bleomycin sulfate (Blenoxane®), busulfan (Myleran®), carboplatin (Paraplatin®), carmustine (BCNU®, Gliadel®, BiCNU®), celecoxib (Celebrex®), cetuximab (IMC-C225 or Erbitux™)), cisplatin (platinum analogs, Platinol®), chlorambucil (Leukeran®), cladribine (2-chlorodeoxyadenosine; "2-CDA"; Leustatin®), cyclophosphamide (Cytoxan®), cyclosporin A, cytarabine (ara-C;Cytosar-U®), daunorubicin (Cerubidine®), dexamethasone, docetaxel, doxorubicin (Doxil®, Rubex® or Adriamycin®), epirubicin (Pharmorubicin®), estramustine, estramustine phosphate (Emcyt®), etoposide (VePesid® or VP-16®), exemestane (Aromasin®), floxuridine (FUDR®), 5-fluorouracil, fludarabine phosphate (Fludara®), gemcitabine HCL (Gemzar®), hydroxyurea (Hydrea®), hexamethylmelamine, idarubicin (Idamycin®), ifosamide (Ifex®), alfa-2a interferon, irinotecan (CPT-11, or Camptosar®), letrozole (Femara®), leucovorin, megestrol (Megace®), methotrexate, methotrexate sodium plus 6-mercaptopurine (6-MP; Purinethol®), mitomycin C, mitoxantrone (Novantrone®), nitrosureas, oxaliplatin, oxorubicin, paclitaxel (Taxol®, Taxotere®, Tamoxifen®, Nolvadex®), pentostatin (2-deoxycoformycin; "DCF"; Nipent®), prednisone, procarbazine, raloxifene (Evista®), raltitrexed (Tomudex®), retinoic acid (ATRA), temozolomide (Temodar®), 6-thioguanine (Tabloid®), thiotepa (Thioplex®), topotecan (Hycamtin®), toremifene (Fareston®), trastuzumab (Herceptin®), valrubicin (Valstar™), vinblastine, vincristine, vincristine sulfate (Oncovin®), and/or vinblastine sulfate (Velban®). The biological composition can also be used after other regimen(s) of treatment is concluded.

The subject may be one who has not yet been diagnosed with cancer but are predisposed to or at high risk of developing as a result of genetic factors and/or environmental factors. The subject may also be one who displays

characteristics that are associated with a high risk of cancer such as nodules detected by computer tomographic scanning or suspect cells in biopsy and/or body fluids.

Depending on the subject, the therapeutic and healthful benefits range from inhibiting or retarding the growth of the cancer and/or the spread of the cancer to other parts of the body (i.e., metastasis), palliating the symptoms of the cancer, improving the probability of survival of the subject with the cancer, prolonging the life expectancy of the subject, improving the quality of life of the subject, and/or reducing the probability of relapse after a successful course of treatment (e.g., surgery, chemotherapy or radiation).

In particular, the invention provides a method for retarding the growth of cancer cells in a subject, such as a human, comprising administering orally to the subject a biological composition of the invention. The invention also provide a method for prolonging the time of survival of a subject inflicted with cancer preferably a human patient, comprising administering orally to the subject a biological composition of the invention.

The effective dose will vary with the subject treated. The effective dose for the subject will also vary with the condition to be treated and the severity of the condition to be treated. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual subject. In general, the total daily dose range of activated and conditioned yeast cells for a subject inflicted with cancer is from about 10^5 to 10^{11} cells per day; preferably, about 10^8 to 5 x 10^{10} cells per day; more preferably, about 2 x 10^9 cells per day in powder form or 9 x 10^8 to 1 x 10^{10} cells per day in liquid preparations, administered in single or divided doses orally. The length of time for a course of treatment should be at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 7 weeks, at least 10 weeks, at least 13 weeks, at least 15 weeks, at least 20 weeks, at least 6 months, or at least 1 year. It may be necessary to use dosages outside these ranges in some cases as will be apparent to those skilled in the art. In certain embodiments, the oral compositions can be administered for a period of time until the symptoms and/or infection of the patients by the bacteria and viruses are under control, or when the disease has regressed partially or completely. For use as a dietary supplement, the total daily dose range should be from about 10^5 to 10^{11} cells per day; preferably, about 5 x 10^7 to 5 x 10^9 cells per day. The oral compositions can be administered as a dietary supplement for as long as 6 months, or in accordance with recommended

length of use under the Dietary Supplement Health and Education Act (DSHEA) or other government or industry guidelines. Further, it is noted that the nutritionist, dietician, clinician or treating physician will know how and when to interrupt, adjust, or terminate use of the biological composition as a medicament or dietary supplement in conjunction with individual patient response.

The effect of the biological compositions of the invention on development and progression of cancer can be monitored by any methods known to one skilled in the art, including but not limited to measuring: a) changes in the size and morphology of the tumor using imaging techniques such as a computed tomographic (CT) scan or a sonogram; and b) changes in levels of biological markers of risk for cancer.

8. Formulations

The biological compositions of the present invention comprise activated and conditioned live yeast cells prepared as described above, as active ingredient, and can optionally contain a pharmaceutically acceptable carrier or excipient, and/or other ingredients provided that these ingredients do not kill or inhibit the yeast cells. Other ingredients that can be incorporated into the biological compositions of the present invention, may include, but are not limited to, herbs (including traditional Chinese medicine products), herbal extracts, vitamins, amino acids, metal salts, metal chelates, coloring agents, flavor enhancers, preservatives, and the like.

Any dosage form may be employed for providing the subject with an effective dosage of the oral composition. Dosage forms include tablets, capsules, dispersions, suspensions, solutions, and the like. In one embodiment, compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, or tablets, each containing a predetermined amount of activated and conditioned yeast cells, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation. Such products can be used as pharmaceuticals or dietary supplements, depending on the dosage and circumstances of its use.

The oral compositions of the present invention may additionally include binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); binders or fillers (e.g., lactose, pentosan, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets or capsules can be coated by methods well known in the art.

Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. The temperature of the liquid used to reconstitute the dried product should be less than 65°C. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). As described below, the preparations can also be made to resemble foods or beverages, containing buffer salts, flavoring, coloring and sweetening agents as appropriate. In certain embodiments, the oral composition is a cell suspension comprising about 10^3 to 10^{10} cells per ml. The oral composition can be produced by diluting or concentrating the yeast culture medium produced by the method set forth herein. In less preferred embodiments, the oral composition is a cell suspension containing about 10^3 to 10^6 cells per ml. In more preferred embodiments, the oral composition is a cell suspension containing greater than about 10^6 to 10^{10} cells per ml. In most preferred embodiments, the oral composition is a cell suspension containing about 10^6 to 5×10^8 cells per ml. The oral composition can be formulated as a health drink and packaged in liquid containers, each containing a predetermined amount of the liquid yeast culture. Standard methods of quality control and packaging are applied to produce in one embodiment of the invention, oral compositions packaged in liquid containers each comprising about 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 10 ml, 15 ml, 20 ml, 30 ml, 40 ml, 50 ml, 75 ml, 100 ml, 150 ml, 200 ml, 250 ml, 500 ml, 750 ml, or 1,000 ml of the live yeast cells. The number of container to be taken each day to obtain the total daily dose in a subject depends on the number of activated and conditioned yeast cells contained within each container. For example, a container

may comprise 50 ml of liquid with 10^7 cells per ml and when a total daily dose of about 2 x 10^9 cells per day is desired, a subject can drink 4 containers per day to obtain the desired total daily dose.

Generally, because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers as described above are employed. In a preferred embodiment, the composition is a capsule. The capsules can be formulated by any commercially available methods. In certain embodiments, the composition is a capsule containing 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 40 mg, 50 mg, 75 mg, 100 mg, 150 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1.0 gram, 1.25 gram, 1.5 gram, or 2.0 gram of live yeast cells in powder form. The powder in the capsule comprises about 10^5 to about 10^{11} cells per gram; more preferably, about 10^8 to 5 x 10^{10} cells per gram; and most preferably, about 5 x 10^8 cells per gram. The number of capsule to be taken each day to obtain the total daily dose in a subject depends on the number of activated and conditioned yeast cells contained within each capsule. For example, a capsule may comprise about 500 mg of powder with 5 x 10^8 cells per gram. To achieve a total daily dose of about 2 x 10^9 cells per day, a subject can take two capsules at a time for four times per day.

In another embodiment, the biological compositions comprising activated and conditioned yeast cells can be added directly to foods so that an effective amount of yeast cells is ingested during normal meals. Any methods known to those skilled in the art may be used to add to or incorporate the biological compositions into natural or processed foods, provided that the activated and conditioned yeast cells remain viable. Preferably, the nutritional compositions of the invention are made and stored under conditions, such as temperature, from about 0°C to 4°C. As used herein, the term "food" broadly refers to any kind of material, liquid or solid, that is used for nourishing an animal, and for sustaining normal or accelerated growth of an animal including humans. Many types of food products or beverages, such as but not limited to, fruit juice, herbal extracts, tea-based beverages, dairy products, soybean product (e.g., tofu), and rice products, can be used to form nutritional compositions comprising the activated and conditioned yeast cells of the invention.

9. Preferred Embodiments

The invention can be further defined by reference to the following preferred embodiments. The use of the current invention for treatment of eighteen (18) commonly occurring cancers is set forth in detail below. However, it is to be understood that these embodiments are provided for illustrative purpose only and thus should not be interpreted to limit the scope of the current invention.

Strains of Yeast Cells

The following yeast cell strains were used to prepare the biological compositions that were administered to the animals used in the respective human cancer type models.

Table 3 Exemplary Strains of Yeast Cells for Treatment of Cancers

Cancer Type Model	Yeast Cell Strain
Lung Cancer	*Saccharomyces cerevisiae* Hansen strain IFFI1345
Nasopharyngeal Cancer	*Saccharomyces carlsbergensis* Hansen strain AS2.116
Esophageal Cancer	*Saccharomyces cerevisiae* Hansen strain AS2.375
Stomach Cancer	*Saccharomyces cerevisiae* Hansen strain AS2.14
Colorectal Cancer	*Saccharomyces cerevisiae* Hansen strain AS2.1396
Pancreatic Cancer	*Saccharomyces cerevisiae* Hansen strain IFFI 1413
Liver Cancer	*Saccharomyces cerevisiae* Hansen strain AS2.503
Testicular Cancer	*Saccharomyces cerevisiae* Hansen strain AS2.182
Prostate Cancer	*Saccharomyces carlsbergensis* Hansen strain AS2.440
Ovarian Cancer	*Saccharomyces cerevisiae* Hansen strain AS2.502
Breast Cancer	*Saccharomyces carlsbergensis* Hansen strain AS2.441
Cervical Cancer	*Saccharomyces carlsbergensis* Hansen strain AS2.444
Uterine Cancer	*Saccharomyces carlsbergensis* Hansen strain AS2.605
Kidney Cancer	*Saccharomyces carlsbergensis* Hansen strain AS2.189
Bladder Cancer	*Saccharomyces cerevisiae* Hansen strain AS2.4
Brain Cancer	*Saccharomyces cerevisiae* Hansen strain AS2.501
Lymphoma	*Saccharomyces cerevisiae* Hansen strain AS2.562
Leukemia	*Saccharomyces cerevisiae* Hansen strain AS2.11

Activation of Yeast Cells

The following electromagnetic (EM) fields can be used to activate the yeast cells to prepare the biological compositions of the invention. The yeast cells as set forth above in Table 3 were cultured in a liquid medium comprising one or more of sucrose or glucose (10-30 g), soluble starch (10 g), mannitol (10 g), vitamin A (40-60 μg), vitamin B_2 (40-50 μg), vitamin B_3 (40-60 μg), vitamin B_6 (30-80 μg), vitamin B_{12} (30-60 μg), vitamin C (40-50 μg), vitamin D (20 μg), vitamin H (20-70 μg),

bovine calf serum (30-35 ml), fetal calf serum (25-45 ml), KH_2PO_4 (0.20 g), $MgSO_4.7H_2O$ (0.20-0.25 g), NaCl (0.20-0.30 g), $CaSO_4.2H_2O$ (0.20-0.30 g), $CaCO_3.5H_2O$ (3.0-4.0 g), peptone (2.5 g), and autoclaved water (up to 1,000 ml), and were subjected to a series of at least two, three, four, or five EM fields as set forth below in Table 4 (numbers shown in parentheses are the preferred embodiments) in accordance with the general procedures set forth in the Detailed Description of the Invention.

Table 4

Cancer Type Model	1st EM Field	2nd EM Field	3rd EM Field	4th EM Field	5th EM Field
Lung Cancer	8046-8056 (8051) MHz, 240-300 (293)mV/cm, about 5-25 (15) hrs	8077-8087 (8082) MHz, 226-285 (272)mV/cm, about 16-36 (26) hrs	9907-9917 (9912)MHz, 325-366 (354)mV/cm, about 13-33 (23) hrs	12716-12726 (12721)MHz, 386-412 (398)mV/cm, about 14-34 (24) hrs	12751-12760 (12755) MHz, 295-315 (302)mV/cm, about 5-25 (15) hrs
Nasopha-ryngeal Cancer	9961-9970 (9963) MHz, 250-270 (246)mV/cm, about 12-32 (22) hrs	10951-10960 (10956) MHz, 250-270 (250)mV/cm, about 5-15 (10) hrs	12091-12100 (12098)MHz, 250-270 (263)mV/cm, about 14-34 (24) hrs	12441-12450 (12446)MHz, 240-270 (244)mV/cm, about 5-15 (10) hrs	12750-12760 (12757) MHz, 270-290 (276)mV/cm, about 14-34 (24) hrs
Eso-phageal Cancer	7841-7850 (7846) MHz, 260-280 (272)mV/cm, about 10-30 (19.5) hrs	8921-8930 (8926) MHz, 250-270 (262)mV/cm, about 4-12 (8) hrs	10191-10200 (10192)MHz, 290-310 (293)mV/cm, about 14-34 (24) hrs	11241-11250 (11248)MHz, 270-290 (280)mV/cm, about 5-15 (10) hrs	12651-12660 (12652) MHz, 310-330 (317)mV/cm, about 14-34 (24) hrs
Stomach Cancer	7981-7990 (7987) MHz, 280-300 (286)mV/cm, about 11-31 (21) hrs	9131-9140 (9137) MHz, 280-300 (281)mV/cm, about 4-12 (8) hrs	10171-10180 (10178)MHz, 300-320 (312)mV/cm, about 12-32 (22) hrs	11221-11230 (11224)MHz, 300-320 (310)mV/cm, about 5-15 (10) hrs	12166-12175 (12171) MHz, 320-340 (332)mV/cm, about 10-30 (20) hrs
Colo-rectal Cancer	7941-7950 (7948) MHz, 230-250 (231)mV/cm, about 14-34 (24) hrs	9131-9140 (9135) MHz, 220-240 (221)mV/cm, about 5-15 (10) hrs	9991-10000 (9997)MHz, 250-270 (254)mV/cm, about 12-32 (22) hrs	11141-11150 (11148)MHz, 230-250 (233)mV/cm, about 5-15 (10) hrs	12771-12780 (12778) MHz, 260-280 (266)mV/cm, about 12-32 (22) hrs
Pancreat-ic Cancer	7961-7970 (7967) MHz, 250-270 (264)mV/cm,	10181-10190 (10188) MHz, 250-270 (266)mV/cm,	12276-12285 (12281)MHz, 270-290 (279)mV/cm,	12461-12470 (12466)MHz, 280-300 (286)mV/cm,	12761-12770 (12764) MHz, 300-320 (306)mV/cm,

	about 12-32 (22) hrs	about 5-15 (10) hrs	about 14-34 (24) hrs	about 5-15 (10) hrs	about 8-28 (18) hrs
Liver Cancer	7971-7980 (7976) MHz, 330-350 (335)mV/cm, about 10-30 (20) hrs	8841-8850 (8845) MHz, 300-320 (310)mV/cm, about 5-15 (10) hrs	10151-10160 (10152)MHz, 390-430 (422)mV/cm, about 14-34 (24) hrs	12121-12130 (12126)MHz, 400-420 (416)mV/cm, about 5-15 (10) hrs	12851-12860 (12854) MHz, 420-450 (445)mV/cm, about 14-34 (24) hrs
Testi-cular Cancer	8021-8030 (8028) MHz, 310-330 (327)mV/cm, about 14-34 (24) hrs	9011-9020 (9016) MHz, 310-330 (326)mV/cm, about 5-15 (10) hrs	9936-9945 (9941)MHz, 355-375 (365)mV/cm, about 14-34 (24) hrs	12061-12070 (12066)MHz, 355-375 (350)mV/cm, about 5-15 (10) hrs	12731-12740 (12737) MHz, 380-400 (387)mV/cm, about 14-34 (24) hrs
Prostate Cancer	10161-10170 (10164) MHz, 230-250 (239)mV/cm, about 12-32 (22) hrs	11211-11220 (11218) MHz, 220-240 (232)mV/cm, about 5-15 (10) hrs	12176-12185 (12181)MHz, 250-270 (258)mV/cm, about 12-32 (22) hrs	12536-12545 (12541)MHz, 250-270 (251)mV/cm, about 5-15 (10) hrs	12656-12665 (12661) MHz, 280-300 (283)mV/cm, about 14-34 (24) hrs
Ovarian Cancer	10081-10090 (10088)MHz, 230-250 (243)mV/cm, about 10-30 (20) hrs	11210-11220 (11214) MHz, 210-230 (228)mV/cm, about 4-12 (8) hrs	12141-12150 (12142)MHz, 245-265 (257)mV/cm, about 12-32 (22) hrs	12341-12350 (12346)MHz, 220-240 (216)mV/cm, about 4-12 (8) hrs	12781-12790 (12786) MHz, 280-300 (283)mV/cm, about 14-34 (24) hrs
Breast Cancer	8061-8070 (8066) MHz, 220-240 (236)mV/cm, about 12-32 (22) hrs	10121-10130 (10124) MHz, 210-230 (226)mV/cm, about 4-12 (8) hrs	12241-12250 (12246)MHz, 255-275 (264)mV/cm, about 10-30 (20) hrs	12351-12360 (12355)MHz, 260-280 (275)mV/cm, about 4-12 (8) hrs	12791-12800 (12798) MHz, 270-290 (277)mV/cm, about 12-32 (22) hrs
Cervical Cancer	7821-7830 (7825) MHz, 230-250 (244)mV/cm, about 14-34 (24) hrs	9051-9060 (9056) MHz, 220-240 (238)mV/cm, about 4-12 (8) hrs	10171-10180 (10175)MHz, 250-270 (258)mV/cm, about 12-32 (22) hrs	11896-11905 (11898)MHz, 260-280 (265)mV/cm, about 4-12 (8) hrs	12786-12795 (12792) MHz, 280-300 (283)mV/cm, about 14-34 (24) hrs
Uterine Cancer	10161-10170 (10162)MHz, 220-240 (235)mV/cm, about 14-34 (24) hrs	11521-11530 (11526) MHz, 210-230 (226)mV/cm, about 4-12 (8) hrs	12131-12140 (12132)MHz, 255-275 (266)mV/cm, about 14-34 (24) hrs	12451-12460 (12458)MHz, 260-280 (265)mV/cm, about 4-12 (8) hrs	12696-12705 (12698) MHz, 270-290 (275)mV/cm, about 14-34 (24) hrs
Kidney Cancer	8036-8045 (8042) MHz, 210-230 (223)mV/cm, about 16-36	11061-11070 (11065) MHz, 205-225 (212)mV/cm, about 4-12 (8)	12081-12090 (12087)MHz, 235-255 (245)mV/cm, about 12-32	12361-12370 (12362)MHz, 230-250 (234)mV/cm, about 4-12	12861-12870 (12865) MHz, 260-280 (264)mV/cm, about 16-36

	(26) hrs	hrs	(22) hrs	(8) hrs	(26) hrs
Bladder Cancer	7891-7900 (7895) MHz, 230-250 (246)mV/cm, about 14-34 (24) hrs	10181-10190 (10184) MHz, 220-240 (236)mV/cm, about 4-12 (8) hrs	12146-12155 (12149)MHz, 250-270 (259)mV/cm, about 14-34 (24) hrs	12661-12670 (12665)MHz, 240-270 (246)mV/cm, about 4-12 (8) hrs	12891-12900 (12897) MHz, 280-300 (283)mV/cm, about 14-34 (24) hrs
Brain Cancer	8006-8015 (8011) MHz, 300-320 (315)mV/cm, about 14-34 (24) hrs	9141-9150 (9147) MHz, 300-320 (312)mV/cm, about 4-12 (8) hrs	9936-9945 (9941)MHz, 345-365 (356)mV/cm, about 14-34 (24) hrs	11331-11340 (11335)MHz, 330-350 (332)mV/cm, about 4-12 (8) hrs	12031-12040 (12032) MHz, 370-390 (375)mV/cm, about 14-34 (24) hrs
Lym-phoma	7972-7983 (7977) MHz, 250-300 (282)mV/cm, about 10-30 (20)hrs	7993-8003 (7998) MHz, 250-300 (277)mV/cm, about 10-30 (20)hrs	10024-10034 (10029)MHz, 370-400 (387)mV/cm, about 12-32 (22)hrs	12832-12842 (12837)MHz, 420-450 (442)mV/cm, about 12-32 (22)hrs	12857-12867 (12862) MHz, 310-350 (325)mV/cm, about 6-18 (12)hrs
Leu-kemia	9971-9980 (9978) MHz, 200-220 (217)mV/cm, about 14-34 (24) hrs	11481-11490 (11484) MHz, 190-210 (207)mV/cm, about 4-12 (8) hrs	12191-12200 (12196)MHz, 230-250 (238)mV/cm, about 14-34 (24) hrs	12561-12570 (12565)MHz, 240-270 (244)mV/cm, about 4-12 (8) hrs	12611-12620 (12612) MHz, 260-280 (262)mV/cm, about 14-34 (24) hrs

Condition of Yeast Cells

The following electromagnetic (EM) fields can be used to condition the activated yeast cells in the biological compositions of the invention. The activated yeast cells were conditioned by culturing in a liquid medium comprising wild hawthorn juice and gastric juice of a mammal, and subjected to at least one of the two EM fields as shown below in Table 5 (numbers shown in parentheses are for the preferred embodiments) by following the general procedures set forth in the Detailed Description of the Invention.

Table 5

Type of Cancer	1st EM Field	2nd EM Field
Lung Cancer	12716-12726 (12721)Hz, 380-420 (386) mV/cm, about 30-50 (40)hrs	12750-12760 (12755)MHz, 280-320 (295) mV/cm, about 12-32 (22)hrs
Nasopharyngeal Cancer	12441-12450 (12446)Hz, 260-300 (275) mV/cm, about 6-18 (12) hrs	12751-12760 (12757)MHz, 290-310 (303) mV/cm, about 26-46 (36) hrs
Esophageal	11241-11250 (11248)Hz,	12651-12660 (12652)MHz,

Cancer	280-320 (312) mV/cm, about 6-18 (12) hrs	320-340 (333) mV/cm, about 27-47 (37) hrs
Stomach Cancer	11221-11230 (11224)Hz, 300-320 (315) mV/cm, about 6-18 (12) hrs	12166-12175 (12171)MHz, 340-360 (346) mV/cm, about 28-48 (38) hrs
Colorectal Cancer	11141-11150 (11148)Hz, 230-250 (242) mV/cm, about 6-18 (12) hrs	12771-12780 (12778)MHz, 290-310 (293) mV/cm, about 26-46 (36) hrs
Pancreatic Cancer	12461-12470 (12466)Hz, 300-320 (306) mV/cm, about 6-18 (12) hrs	12761-12770 (12764)MHz, 300-330 (323) mV/cm, about 22-42 (32) hrs
Liver Cancer	12121-12130 (12126)Hz, 420-440 (422) mV/cm, about 6-18 (12) hrs	12851-12860 (12854)MHz, 400-450 (437) mV/cm, about 30-50 (40) hrs
Testicular Cancer	12061-12070 (12066)Hz, 320-340 (326) mV/cm, about 5-15 (10) hrs	12731-12740 (12737)MHz, 340-360 (346) mV/cm, about 24-44 (34) hrs
Prostate Cancer	12536-12545 (12541)Hz, 260-280 (265) mV/cm, about 5-15 (10) hrs	12656-12665 (12661)MHz, 280-300 (298) mV/cm, about 22-42 (32) hrs
Ovarian Cancer	12341-12350 (12346)Hz, 280-300 (284) mV/cm, about 4-12 (8) hrs	12781-12790 (12786)MHz, 290-310 (306) mV/cm, about 32-52 (42) hrs
Breast Cancer	12351-12360 (12355)Hz, 280-300 (286) mV/cm, about 6-18 (12) hrs	12791-12800 (12798)MHz, 280-300 (298) mV/cm, about 28-48 (38) hrs
Cervical Cancer	11896-11905 (11898)Hz, 280-300 (288) mV/cm, about 6-18 (12) hrs	12786-12795 (12792)MHz, 290-310 (307) mV/cm, about 24-44 (34) hrs
Uterine Cancer	12451-12460 (12458)Hz, 280-300 (284) mV/cm, about 6-18 (12) hrs	12696-12705 (12698)MHz, 280-300 (297) mV/cm, about 24-44 (34) hrs
Kidney Cancer	12361-12370 (12362)Hz, 245-265 (250) mV/cm, about 6-18 (12) hrs	12861-12870 (12865)MHz, 265-285 (282) mV/cm, about 32-52 (42) hrs
Bladder Cancer	12661-12670 (12665)Hz, 255-275 (256) mV/cm, about 6-18 (12) hrs	12891-12900 (12897)MHz, 270-300 (293) mV/cm, about 24-44 (34) hrs
Brain Cancer	11331-11340 (11335)Hz, 300-320 (306) mV/cm, about 6-18 (12) hrs	12031-12040 (12032)MHz, 305-325 (322) mV/cm, about 24-44 (34) hrs
Lymphoma	12832-12842 (12837)Hz, 380-400 (391) mV/cm, about 29-49 (39)hrs	12857-12867 (12862)MHz, 300-350 (315) mV/cm, about 10-30 (20)hrs
Leukemia	12561-12570 (12565)Hz, 260-290 (265) mV/cm, about 6-18 (12) hrs	12611-12620 (12612)MHz, 260-280 (278) mV/cm, about 29-49 (39) hrs

Large-scale Manufacturing of Yeast Cells

The following EM fields (numbers shown in parentheses are for the preferred embodiments) can be applied in accordance with the steps set forth in the Detailed Description of the Invention to mass produce the activated and conditioned yeast cells that were used to prepare the biological compositions that were administered to the animals used in the respective human cancer type.

Table 6

Cancer Type	First Container (5)		Second Container (6)		Third Container (7)	
	1st EM Field	2nd EM Field	1st EM Field	2nd EM Field	1st EM Field	2nd EM Field
Lung Cancer	12,716-12,726 (12,721) MHz, 210-450 (422) mV/cm, about 5-25 (15) hrs	12,750-12,760 (12,755) MHz, 145-400 (388) mV/cm, about 6-18 (12) hrs	12,716-12,726 (12,721) MHz, 320-350 (341) mV/cm, about 6-18 (12) hrs	12,750-12,760 (12,755) MHz, 220-250 (231) mV/cm, about 5-15 (10) hrs	12,716-12,726 (12,721) MHz, 210-250 (234) mV/cm, about 12-32 (22) hrs	12,750-12,760 (12,755) MHz, 145-165 (154) mV/cm, about 6-18 (12) hrs
Nasopharyngeal Cancer	12,441-12,450 (12,446) MHz, 310-330 (312) mV/cm, about 5-15 (10) hrs	12,751-12,760 (12,757) MHz, 310-330 (327) mV/cm, about 6-18 (12) hrs	12,441-12,450 (12,446) MHz, 240-255 (250) mV/cm, about 5-15 (10) hrs	12,751-12,760 (12,757) MHz, 330-350 (343) mV/cm, about 6-18 (12) hrs	12,441-12,450 (12,446) MHz, 215-235 (220) mV/cm, about 6-18 (12) hrs	12,751-12,760 (12,757) MHz, 240-260 (251) mV/cm, about 14-34 (24) hrs
Esophageal Cancer	11,241-11,250 (11,248) MHz, 310-330 (322) mV/cm, about 4-12 (8) hrs	12,651-12,660 (12,652) MHz, 330-360 (356) V/cm, about 5-15 (10) hrs	11,241-11,250 (11,248) MHz, 320-340 (332) mV/cm, about 4-12 (8) hrs	12,651-12,660 (12,652) MHz, 340-360 (357) mV/cm, about 5-15 (10) hrs	11,241 to 11,250 (11,248) MHz, 210-230 (211) mV/cm, about 6-18 (12) hrs	12,651-12,660 (12,652) MHz, 230-250 (238) mV/cm, about 14-34 (24) hrs
Stomach Cancer	11,221-11,230 (11,224) MHz, 310-330 (322) mV/cm, about 4-12 (8) hrs	12,166-12,175 (12,171) MHz, 360-380 (377) mV/cm, about 6-18 (12) hrs	11,221-11,230 (11,224) MHz, 340-360 (346) mV/cm, about 4-12 (8) hrs	12,166-12,175 (12,171) MHz, 380-410 (404) mV/cm, about 4-24 (14) hrs	11,221-11,230 (11,224) MHz, 215 to 235 (222) mV/cm, about 6-18 (12) hrs	12,166-12,175 (12,171) MHz, 230-250 (242) mV/cm, about 12-32 (22) hrs
Colorectal	11,141-	12,771-	11,141-	12,771-	11,141-	12,771-

Cancer	11,150 (11,148) MHz, 280-300 (286) mV/cm, for about 4-12 (8) hrs	12,780 (12,778) MHz, 310-330 (322) mV/cm, about 5-15 (10) hrs	11,150 (11,148) MHz, 280-300 (292) mV/cm, about 4-12 (8) hrs	12,780 (12,778) MHz, 330-350 (332) mV/cm, about 6-18 (12) hrs	11,150 (11,148) MHz, 180-200 (188) mV/cm, about 6-18 (12) hrs	12,780 (12,778) MHz, 230-250 (236) mV/cm, about 14-34 (24) hrs
Pancreatic Cancer	12,461-12,470 (12,466) MHz, 310-330 (328) mV/cm, about 4-12 (8) hrs	12,761-12,770 (12,764) MHz, 330-350 (338) mV/cm, about 6-18 (12) hrs	12,461-12,470 (12,466) MHz, 330-340 (334) mV/cm, about 4-12 (8) hrs	12,761-12,770 (12,764) MHz, 350-370 (362) mV/cm, about 6-18 (12) hrs	12,461-12,470 (12,466) MHz, 220-240 (238) mV/cm, about 6-18 (12) hrs	12,761-12,770 (12,764) MHz, 250-270 (263) mV/cm, about 14-34 (24) hrs
Liver Cancer	12,121-12,130 (12,126) MHz, 400-420 (412) mV/cm, for about 5-15 (10) hrs	12,851-12,860 (12,854) MHz, 420-450 (436) mV/cm, about 4-24 (14) hrs	12,121-12,130 (12,126) MHz, 300-320 (308) mV/cm, about 5-15 (10) hrs	12,851-12,860 (12,854) MHz, 320-350 (325) mV/cm, about 6-18 (12) hrs	12,121 to 12,130 (12,126) MHz, 210-230 (211) mV/cm, about 5-15 (10) hrs	12,851-12,860 (12,854) MHz, 230-250 (235) mV/cm, about 14-34 (24) hrs
Testicular Cancer	12,061-12,070 (12,066) MHz, 320-340 (334) mV/cm, about 5-15 (10) hrs	12,731-12,740 (12,737) MHz, 350-370 (367) mV/cm, about 6-18 (12) hrs	12,061-12,070 (12,066) MHz, 330-350 (346) mV/cm, about 5-15 (10) hrs	12,731-12,740 (12,737) MHz, 400-420 (412) mV/cm, about 6-18 (12) hrs	12,061-12,070 (12,066) MHz, 340-360 (346) mV/cm, about 6-18 (12) hrs	12,731-12,740 (12,737) MHz, 400-420 (417) mV/cm, about 14-34 (24) hrs
Prostate Cancer	12,536-12,545 (12,541) MHz, 250-270 (260) mV/cm, about 4-12 (8) hrs	12,656-12,665 (12,661) MHz, 320-340 (322) mV/cm, about 6-18 (12) hrs	12,536-12,545 (12,541) MHz, 260-280 (267) mV/cm, about 4-12 (8) hrs	12,656-12,665 (12,661) MHz, 320-340 (338) mV/cm, about 6-18 (12) hrs	12,536-12,545 (12,541) MHz, 170-190 (188) mV/cm, about 6-18 (12) hrs	12,656-12,665 (12,661) MHz, 230-250 (245) mV/cm, about 14-34 (24) hrs
Ovarian Cancer	12,341-12,350 (12,346) MHz, 300-320 (311)	12,781-12,790 (12,786) MHz, 320-340 (322)	12,341-12,350 (12,346) MHz, 320-340 (323)	12,781-12,790 (12,786) MHz, 330-350 (342)	12,341-12,350 (12,346) MHz, 230-240 (236)	12,781-12,790 (12,786) MHz, 220-240 (234)

	mV/cm, about 4-12 (8) hrs	mV/cm, about 6-18 (12) hrs	mV/cm, about 4-12 (8) hrs	mV/cm, about 6-18 (12) hrs	mV/cm, about 5-15 (10) hrs	mV/cm, about 12-32 (22) hrs
Breast Cancer	12,351-12,360 (12,355) MHz, 300-320 (312) mV/cm, about 4-12 (8) hrs	12,791-12,800 (12,798) MHz, 310-330 (317) mV/cm, about 6-18 (12) hrs	12,351-12,360 (12,355) MHz, 320-340 (322) mV/cm, about 4-12 (8) hrs	12,791-12,800 (12,798) MHz, 330-350 (348) mV/cm, about 6-18 (12) hrs	12,351-12,360 (12,355) MHz, 210-230 (211) mV/cm, about 6-18 (12) hrs	12,791-12,800 (12,798) MHz, 210-230 (226) mV/cm, about 14-34 (24) hrs
Cervical Cancer	11,896-11,905 (11,898) MHz, 290-310 (305) mV/cm, about 4-12 (8) hrs	12,786-12,795 (12,792) MHz, 320-340 (322) mV/cm, about 6-18 (12) hrs	11,896-11,905 (11,898) MHz, 310-330 (315) mV/cm, about 4-12 (8) hrs	12,786-12,795 (12,792) MHz, 320-340 (337) mV/cm, about 6-18 (12) hrs	11,896-11,905 (11,898) MHz, 210-230 (228) mV/cm, about 6-18 (12) hrs	12,786-12,795 (12,792) MHz, 240-260 (255) mV/cm, about 14-34 (24) hrs
Uterine Cancer	12,451-12,460 (12,458) MHz, 300-320 (316) mV/cm, about 4-12 (8) hrs	12,696-12,705 (12,698) MHz, 320-340 (327) mV/cm, about 6-18 (12) hrs	12,451-12,460 (12,458) MHz, 320-340 (328) mV/cm, about 4-12 (8) hrs	12,696-12,705 (12,698) MHz, 330-350 (346) mV/cm, about 6-18 (12) hrs	12,451-12,460 (12,458) MHz, 250-270 (262) mV/cm, about 6-18 (12) hrs	12,696-12,705 (12,698) MHz, 250-270 (262) mV/cm, about 14-34 (24) hrs
Kidney Cancer	12,361-12,370 (12,362) MHz, 245-265 (262) mV/cm, about 4-12 (8) hrs	12,861-12,870 (12,865) MHz, 300-320 (302) mV/cm, about 6-18 (12) hrs	12,361-12,370 (12,362) MHz, 265-285 (270) mV/cm, about 4-12 (8) hrs	12,861-12,870 (12,865) MHz, 305-325 (321) mV/cm, about 6-18 (12) hrs	12,361-12,370 (12,362) MHz, 195-215 (212) mV/cm,, about 6-18 (12) hrs	12,861-12,870 (12,865) MHz, 245-265 (262) mV/cm, about 14-34 (24) hrs
Bladder Cancer	12,661-12,670 (12,665) MHz, 240-260 (256) mV/cm, about 4-12 (8) hrs	12,891-12,900 (12,897) MHz, 320-340 (322) mV/cm, about 6-18 (12) hrs	12,661-12,670 (12,665) MHz, 260-280 (266) mV/cm, about 4-12 (8) hrs	12,891-12,900 (12,897) MHz, 320-340 (332) mV/cm, about 6-18 (12) hrs	12,661-12,670 (12,665) MHz, 170-190 (188) mV/cm, about 6-18 (12) hrs	12,891-12,900 (12,897) MHz, 230-250 (247) mV/cm, about 14-34 (24) hrs
Brain Cancer	11,331-11,340	12,031-12,040	11,331-11,340	12,031-12,040	11,331-11,340	12,031-12,040

	(11,335) MHz, 300-320 (318) mV/cm, about 4-12 (8) hrs	(12,032) MHz, 350-370 (357) mV/cm, about 6-18 (12) hrs	(11,335) MHz, 330-350 (335) mV/cm, about 4-12 (8) hrs	(12,032) MHz, 390-410 (406) mV/cm, about 6-18 (12) hrs	(11,335) MHz, 335-335 (350) mV/cm, about 6-18 (12) hrs	(12,032) MHz, 390-420 (415) mV/cm, about 14-34 (24) hrs
Lymphoma	12,832-12,842 (12,837) MHz, 420-450 (442) mV/cm, about 6-18 (12) hrs	12,857-12,867 (12,862) MHz, 340-380 (350) mV/cm, about 5-15 (10) hrs	12,832-12,842 (12,837) MHz, 330-350 (341) mV/cm, about 4-24 (14) hrs	12,857-12,867 (12,862) MHz, 210-240 (224) mV/cm, about 5-15 (10) hrs	12,832-12,842 (12,837) MHz, 230-250 (244) mV/cm, about 12-32 (22) hrs	12,857-12,867 (12,862) MHz, 120-150 (137) mV/cm, about 6-18 (12) hrs
Leukemia	12,561-12,570 (12,565) MHz, 255- 275 (270) mV/cm, about 4-12 (8) hrs	12,611-12,620 (12,612) MHz, 290-310 (296) mV/cm, about 6-18 (12) hrs	12,561-12,570 (12,565) MHz, 270-290 (277) mV/cm, about 4-12 (8) hrs	12,611-12,620 (12,612) MHz, 290-320 (318) mV/cm, about 6-18 (12) hrs	12,561-12,570 (12,565) MHz, 170-190 (184) mV/cm, about 6-18 (12) hrs	12,611-12,620 (12,612) MHz, 250-270 (267) mV/cm, about 14-34 (24) hrs

Lung Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from lung cancer. As used herein, the term "lung cancer" includes but is not limited to squamous cell carcinoma, small cell carcinoma, adenocarcinoma, large cell carcinoma, carcinoid tumor, and mesothelioma. In a specific embodiment, the biological composition can ameliorate, reduce, manage, or eliminate the symptoms associated with lung cancer, such as cough, shortness of breath, wheezing, chest pain, hemoptysis (bloody, coughed-up sputum), loss of appetite, weight loss, pneumonia (inflammation of the lungs), weakness, chills, swallowing difficulties, speech difficulties or changes (*e.g.*, hoarseness), finger/nail abnormalities (*e.g.*, "clubbing," or overgrowth of the fingertip tissue), skin paleness or bluish discoloration, muscle contractions or atrophy (shrinkage), joint pain or swelling, facial swelling or paralysis, eyelid drooping, bone pain/tenderness, and breast development in men.

According to the invention, the biological composition useful for treatment of lung cancer comprises activated and conditioned *Saccharomyces cerevisiae* Hansen strain IFFI1345 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 8,051 MHz and a field strength of 293 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 8,082 MHz and a field strength of 272 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 9,912 MHz and a field strength of 354 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,721 MHz and a field strength of 398 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,755 MHz and a field strength of 302 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,721 MHz and a field strength of 386 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,755 MHz and a field strength of 295 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μg of vitamin B_2, 30 μg of vitamin B_6, 30 μg of vitamin B_{12}, 0.20 g of KH_2PO_4, 0.22 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.30 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,721 MHz and a field strength in the range of 210 to 450 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 422 mV/cm, 341 mV/cm, and 234 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,755 MHz and a field strength in the range of 145 to 400 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 388 mV/cm, 231 mV/cm, and 154 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Nasopharyngeal Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from nasopharyngeal cancer. As used herein, the term "nasopharyngeal cancer" includes but is not limited to squamous cell carcinoma, nonkeratinizing carcinomas, undifferentiated carcinomas and keratinizing carcinoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms of the cancer, improving the probability of survival of the subject with the cancer, prolonging the life expectancy of the subject, improving the quality of life of the subject, and/or reducing the probability of relapse after a successful course of treatment (*e.g.*, surgery, chemotherapy or radiation). The symptoms associated with nasopharyngeal cancer include neck mass, hearing loss, ipsilateral serous otitis, hearing loss, nasal obstruction, frank epistaxis, purulent or bloody rhinorrhea, and facial neuropathy or facial nerve palsies.

According to the invention, the biological composition useful for treatment of nasopharyngeal cancer comprises activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.116 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 9,963 MHz and a field strength of 246 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 10,956 MHz and a field strength of 250 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,098 MHz and a field strength of 263 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,446 MHz and a field strength of 244 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,757 MHz and a field strength of 276 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,446 MHz and a field strength of 275 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,757 MHz and a field strength of 303 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μg of vitamin B_6, 40 μg of vitamin B_{12}, 70 μg of vitamin H, 25 ml of fetal calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,446 MHz and a field strength in the range of 215 to

330 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 312 mV/cm, 250 mV/cm, and 220 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,757 MHz and a field strength in the range of 240 to 350 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 327 mV/cm, 343 mV/cm, and 251 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Esophageal Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from esophageal cancer. As used herein, the term "esophageal cancer" includes but is not limited to squamous cell carcinoma, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, carcinosarcoma, pseudosarcoma, sarcomas, melanoma, plasmacytoma, verrucous carcinoma and oat cell carcinoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with esophageal cancer include dysphagia, difficulty swallowing solids or liquids, regurgitation of food, heartburn, vomiting blood and chest plan unrelated to eating.

According to the invention, the biological composition useful for treatment of esophageal cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain AS2.375 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,846 MHz and a field strength of 272 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 8,926 MHz and a field strength of 262 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 10,192 MHz and a field strength of 293 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 11,248 MHz and a field strength of 280 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,652 MHz and a field strength of 317 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 11,248 MHz and a field strength of 312 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,652 MHz and a field strength of 333 mV/cm.

Preferably, the first liquid medium comprises 10 g of sucrose or glucose, 10 g of soluble starch, 50 μg of vitamin B_2, 50 μg of vitamin B_6, 20 μg of vitamin B_{12}, 35 ml of fetal calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 11,248 MHz and a field strength in the range of 210 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 332 mV/cm, and 211 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,652 MHz and a field strength in the range of 230 to 360 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 356 mV/cm, 357 mV/cm, and 238 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Stomach Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from stomach cancer. As used herein, the term "stomach cancer" includes but is not limited to malignant cancer such as adenocarcinoma, papillary adenocarcinoma, tubular adenocarcinoma, mucinous adenocarcinoma, signet ring cell carcinoma, adenosquamous carcinoma, carcinoid tumor, mixed carcinoid-adenocarcinoma, small cell carcinoma (M80413), and undifferentiated carcinoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with stomach cancer include loss of appetite, difficulty in swallowing, vague fullness, vomiting blood, abdominal pain, belching, breath odor, excessive gas and flatus, heartburn, weight loss, and a decline in general health.

According to the invention, the biological composition useful for treatment of stomach cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain AS2.14 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,987 MHz and a field strength of 286 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 9,137 MHz and a field strength of 281 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 10,178 MHz and a field strength of 312 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 11,224 MHz and a field strength of 310 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,171 MHz and a field strength of 332 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency 11,224 MHz and a field strength of 315 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency 12,171 MHz and a field strength of 346 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μg of vitamin B_3, 30 μg of vitamin B_6, 50 μg of vitamin C, 25 ml of fetal calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 11,224 MHz and a field strength in the range of 215 to 360 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 346 mV/cm, and 222 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,171 MHz and a field strength in the range of 230 to 410 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 377 mV/cm, 404 mV/cm, and 242 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Colorectal Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from colorectal cancer. As used herein, the term "colorectal cancer" includes but is not limited to squamous cell (epidermoid) carcinomas, cloacogenic

(basaloid transitional cell) tumors, and adenocarcinomas. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with colorectal cancer include constipation, blood in the stool, unexplained anemia, abdominal pain and tenderness in the lower abdomen, intestinal obstruction, weight loss with no known reason, stools narrower than usual, constant tiredness, and anal lump.

According to the invention, the biological composition useful for treatment of colorectal cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain AS2.1396 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,948 MHz and a field strength of 231 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 9,135 MHz and a field strength of 221 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 9,997 MHz and a field strength of 254 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 11,148 MHz and a field strength of 233 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,778 MHz and a field strength of 266 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 11,148 MHz and a field strength of 242 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,778 MHz and a field strength of 293 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 50 μg of vitamin B_6, 50 μg of vitamin B_{12}, 30 ml of fetal calf serum, 60 μg of vitamin A, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 11,148 MHz and a field strength in the range of 180 to 300 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 286 mV/cm, 292 mV/cm, and 188 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,778 MHz and a field strength in the range of 230 to 350 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 332 mV/cm, and 236 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Pancreatic Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from pancreatic cancer. As used herein, the term "pancreatic cancer" includes but is not limited to adenocarcinomas, acinar cell carcinoma, cystadenocarcinoma (mucinous), adenosquamous carcinoma, solid microglandular carcinoma, carcinoid, sarcoma, and malignant lymphoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with pancreatic cancer include abdominal pain, unexpected weight loss, nausea, loss of appetite, weight loss, digestive problems, jaundice, or yellowing of the skin, restlessness, loss of energy, irritability, sweating, tremor, drowsiness and severe confusion.

According to the invention, the biological composition useful for treatment of pancreatic cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain IFFI 1413 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,967 MHz and a field strength of 264 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 10,188 MHz and a field strength of 266 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,281 MHz and a field strength of 279 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,466 MHz and a field strength of 286 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,764 MHz and a field strength of 306 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,466 MHz and a field strength of 306 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,764 MHz and a field strength of 323 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 50 μg of vitamin B_3, 30 μg of vitamin B_{12}, 20 μg of vitamin H, 40 ml of fetal calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,466 MHz and a field strength in the range of 220 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 328 mV/cm, 334 mV/cm, and 238 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,764 MHz and a field strength in the range of 250 to 370 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 338 mV/cm, 362 mV/cm, and 263 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Liver Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from liver cancer. As used herein, the term "liver cancer" includes but is not limited to hepatocellular carcinoma, cholangiocarcinoma, mixed hepatocellular cholangiocarcinoma, angiosarcoma, fibrolamellar, cystadenoma, and epithelioid hemangioendothelioma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with liver cancer include general malaise as well as pain and tenderness, unexplained weight loss, persistent lack of appetite, fever of unknown origin, limb weakness, sensory loss, persistent abdominal pain, immature feeling of fullness, swelling of the abdominal are with or without breathing difficulties, sudden jaundice, and liver enlargement or a mass that can be felt in the liver area.

According to the invention, the biological composition useful for treatment of liver cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain AS2.503 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a at 7,976 MHz and a field strength of 335 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 8,845 MHz and a field strength of 310 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 10,152 MHz and a field strength of 422 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,126 MHz and a field strength of 416 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,854 MHz and a field strength of 445 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,126 MHz and a field strength of 422 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,854 MHz and a field strength of 437 mV/cm.

Preferably, the first liquid medium comprises 30 g of sucrose or glucose, 40 μg of vitamin B_6, 30 μg of vitamin H, 40 μg of vitamin A, 30 ml of bovine calf serum, 0.20 g of KH_2PO_4, 0.20 g of $MgSO_4.7H_2O$, 0.20 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 3.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,126 MHz and a field strength in the range of 210 to

420 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 412 mV/cm, 308 mV/cm, and 211 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,854 MHz and a field strength in the range of 230 to 450 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 436 mV/cm, 325 mV/cm, and 235 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Testicular Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from testicular cancer. As used herein, the term "testicular cancer" includes but is not limited to malignant cancer such as seminomas, nonseminomas, choriocarcinoma, embryonal carcinoma, immature teratoma, yolk sac tumors, Leydig and sertoli cell tumors, PNET, leiomyosarcoma, rhabdomyosarcoma, and mesothelioma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with testicular cancer include a painless lump, a hardening or a change (increase or decrease) in size of the testicle, a feeling of heaviness or a sudden collection of fluid in the scrotum, a dull ache in the lower abdomen or in the groin, or pain or discomfort in the scrotum or testicle.

According to the invention, the biological composition useful for treatment of testicular cancer comprises activated and conditioned are *Saccharomyces carlsbergensis* Hansen strain AS2.116 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 8,028 MHz and a field strength in the range of 327 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 9,016 MHz and a field strength in the range of 326 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 9,941 MHz and a field strength in the range of 365 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,066 MHz and a field strength in the range of 350 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,737 MHz and a field strength in the range of 387 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,066 MHz and a field strength in the range of 326 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,737 MHz and a field strength in the range of 346 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 60 μg of vitamin B_{12}, 60 μg of vitamin B_3, 60 μg of vitamin H, 50 μg of vitamin B_6, 30 ml of fetal calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,066 MHz and a field strength in the range of 320 to 360 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 334 mV/cm, 346 mV/cm, and 346 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,737 MHz and a field strength in the range of 350 to

420 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 367 mV/cm, 412 mV/cm, and 417 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Prostate Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from prostate cancer. As used herein, the term "prostate cancer" includes but is not limited to adenocarcinomas. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with prostate cancer include frequent urination (especially at night) or an inability to urinate, trouble starting or holding back urine, pain during ejaculation or urination, a weak or interrupted urine flow, blood in the semen or in the urine, or frequent pain or stiffness in the lower back, hips, or upper thighs.

According to the invention, the biological composition useful for treatment of prostate cancer comprises activated and conditioned are *Saccharomyces carlsbergensis* Hansen strain AS2.440 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 10,164 MHz and a field strength of 239 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 11,218 MHz and a field strength of 232 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,181 MHz and a field strength of 258 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,541 MHz and a field strength of 251 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,661 MHz and a field strength of 283 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,541 MHz and a field strength of 265 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,661 MHz and a field strength of 298 mV/cm.

Preferably, the first liquid medium comprises 10 g of sucrose or glucose, 10 g of mannitol, 80 μg of vitamin B_6, 50 μg of vitamin B_3, 60 μg of vitamin H, 60 μg of vitamin A, 30 ml of bovine calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,541 MHz and a field strength in the range of 170 to 280 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 260 mV/cm, 267 mV/cm, and 188 mV/cm ; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,661 MHz and a field strength in the range of 230 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 338 mV/cm, and 245 mV/cm .

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Ovarian Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from ovarian cancer. As used herein, the term "ovarian cancer" includes but is not limited to serous carcinoma and those arising from the celomic epithelium, specialized stroma, and germ cell layer or unfertilized ovum. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with ovarian cancer include abdominal pain, abdominal swelling, bloating or dyspepsia, pelvic pressure, weight gain or loss, abnormal menstrual cycles, increased abdominal girth, vaginal bleeding, excessive hair, and increased urinary frequency or urgency.

According to the invention, the biological composition useful for treatment of ovarian cancer comprises activated and conditioned are *Saccharomyces cerevisiae* Hansen strain AS2.502 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 10,088 MHz and a field strength of 243 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 11,214 MHz and a field strength of 228 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,142 MHz and a field strength of 257 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,346 MHz and a field strength of 216 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,786 MHz and a field strength of 283 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency 12,346 MHz and a field strength of 284 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,786 MHz and a field strength of 306 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μg of vitamin B_{12}, 30 μg of vitamin B_6, 50 μg of vitamin A, 40 μg of vitamin C, 35 ml of bovine calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,346 MHz and a field strength in the range of 230 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 311 mV/cm, 323 mV/cm, and 236 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,786 MHz and a field strength in the range of 220 to 350 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 342 mV/cm, and 234 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Breast Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from breast cancer. As used herein, the term "breast cancer" includes but is not limited to malignant epithelial cancer such as adenocarcinomas (ductal carcinomas and lobular carcinomas), squamous cell carcinoma, nonkeratinizing carcinomas, undifferentiated carcinomas, and keratinizing carcinoma, ductal carcinoma in situ (DCIS), infiltrating (or invasive) ductal carcinoma (IDC), infiltrating (or invasive) lobular carcinoma (ILC), inflammatory breast cancer, in situ,

lobular carcinoma in situ (LCIS), medullary carcinoma, mucinous carcinoma, phyllodes tumor, tubular carcinoma, and Paget's disease of the nipple. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with breast cancer include breast lump(s), swelling of the skin of the breast, discharge from the nipple, enlargement of the lymph glands of the armpit, nipple erosion or ulceration, diffuse erythema of the breast, and axillary adenopathy.

According to the invention, the biological composition useful for treatment of breast cancer comprises activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.441 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 8,066 MHz and a field strength of 236 mV/cm ;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 10,124 MHz and a field strength of 226 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,246 MHz and a field strength of 264 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,355 MHz and a field strength of 275 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,798 MHz and a field strength of 277 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,355 MHz and a field strength of 286 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,798 MHz and a field strength of 298 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 30 μg of vitamin B_{12}, 50 μg of vitamin B_3, 40 μg of vitamin H, 40 μg of vitamin C, 35 ml of bovine calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,355 MHz and a field strength in the range of 210 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 312 mV/cm, 322 mV/cm, and 211 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,798 MHz and a field strength in the range of 210 to 350 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 317 mV/cm, 348 mV/cm, and 226 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Cervical Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from cervical cancer. As used herein, the term "cervical cancer" includes but is not limited to adenocarcinomas (ductal carcinomas and lobular carcinomas), squamous cell carcinoma, nonkeratinizing carcinomas, undifferentiated carcinomas, and keratinizing carcinoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with cervical cancer include abnormal bleeding, such as between periods or after intercourse, persistent vaginal discharge, which may be pale, watery, pink, brown, blood streaked, or dark and foul-smelling, and discomfort during intercourse.

According to the invention, the biological composition useful for treatment of cervical cancer comprises activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.444 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,825 MHz and a field strength of 244 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 9,056 MHz and a field strength of 238 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 10,175 MHz and a field strength of 258 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 11,898 MHz and a field strength of 265 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,792 MHz and a field strength of 283 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency 11,898 MHz and a field strength of 288 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,792 MHz and a field strength of 307 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μg of vitamin B_{12}, 40 μg of vitamin B_3, 50 μg of vitamin H, 50 μg of vitamin B_6, 45 ml of fetal calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 11,898 MHz and a field strength in the range of 210 to 330 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 305 mV/cm, 315 mV/cm, and 228 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,792 MHz and a field strength in the range of 240 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 337 mV/cm, and 255 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Uterine Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from uterine cancer. As used herein, the term "uterine cancer" includes but is not limited to squamous cell carcinoma, endometrioid carcinoma, adenoacanthoma, adenosquamous carcinoma, papillary serous carcinomas, and clear cell adenocarcinomas. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with uterine cancer include abnormal vaginal and/or uterine spotting or bleeding, such as between periods or after intercourse, white or clear vaginal discharge, difficult or painful urination, discomfort during intercourse, pain or cramping in the pelvic area, and discomfort during intercourse .

According to the invention, the biological composition useful for treatment of uterine cancer comprises activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.605 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 10,162 MHz and a field strength of 235 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 11,526 MHz and a field strength of 226 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,132 MHz and a field strength of 266 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,458 MHz and a field strength of 265 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,698 MHz and a field strength of 275 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,458 MHz and a field strength of 284 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,698 MHz and a field strength of 297 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 60 μg of vitamin B_{12}, 60 μg of vitamin B_3, 60 μg of vitamin H, 50 μg of vitamin B_6, 30 ml of fetal calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,458 MHz and a field strength in the range of 250 to

340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 316 mV/cm, 328 mV/cm, and 262 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,698 MHz and a field strength in the range of 250 to 350 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 327 mV/cm, 346 mV/cm, and 262 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Kidney Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from kidney cancer. As used herein, the term "kidney cancer" includes but is not limited to adenocarcinomas, hypernephroma, renal cell carcinoma, clear cell cancer, and Grawitz's tumor. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with kidney cancer include blood in the urine (hematuria), persistent pain in the area between the ribs and the hip not associated with an injury, a mass in the area of the kidneys, high blood pressure, rapid and unexplained weight loss, persistent feeling of fatigue, fever not caused by a cold or flu, swelling of the legs and ankles, anemia, fatigue, and intermittent fever.

According to the invention, the biological composition useful for treatment of kidney cancer comprises activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.189 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 8,042 MHz and a field strength of 223 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 11,065 MHz and a field strength of 212 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,087 MHz and a field strength of 245 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,362 MHz and a field strength of 234 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,865 MHz and a field strength of 264 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,362 MHz and a field strength of 250 mV/cm m; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,865 MHz and a field strength of 282 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μg of vitamin B_3, 30 μg of vitamin B_6, 60 μg of vitamin A, 20 μg of vitamin H, 50 μg of vitamin C, 30 ml of fetal calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 980 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,362 MHz and a field strength in the range of 195 to 285 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 262 mV/cm, 270 mV/cm, and 212 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,865 MHz and a field strength in the range of 245 to 325

mV/cm, preferably at three fields strengths, *e.g.*, in the order of 302 mV/cm, 321 mV/cm, and 262 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Bladder Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from bladder cancer. As used herein, the term "bladder cancer" includes but is not limited to urothelial carcinoma, transitional cell carcinoma, squamous cell carcinoma, adenocarcinoma, papillomas, flat urothelial carcinomas, and rhabdomyosarcoma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with bladder cancer include microscopic (visible only under a microscope) or gross (visible to the naked eye) hematuria, or blood in the urine, frequent urination, urinary urgency, urinary frequency, painful urinary (dysuria), urinary incontinence, bone pain or tenderness, abdominal pain, anemia, weight loss, and lethargy.

According to the invention, the biological composition useful for treatment of bladder cancer comprises activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.4 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,895 MHz and a field strength of 246 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 10,184 MHz and a field strength of 236 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,149 MHz and a field strength of 259 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,665 MHz and a field strength of 246 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,897 MHz and a field strength of 283 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,665 MHz and a field strength of 256 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,897 MHz and a field strength of 293 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 50 μg of vitamin B_{12}, 30 μg of vitamin B_6, 20 μg of vitamin H, 50 μg of vitamin A, 30 ml of bovine calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,665 MHz and a field strength in the range of 170 to 280 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 256 mV/cm, 266 mV/cm, and 188 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,897 MHz and a field strength in the range of 230 to 340 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 322 mV/cm, 332 mV/cm, and 247 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Brain Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from brain cancer. As used herein, the term "brain cancer" includes but is not limited to astrocytoma, meningioma, colloid cyst, ependymoma, metastatic tumors, choroid plexus papilloma, subependymoma, astrocytoma, glioblastoma, lipoma, oligodendroglioma, sarcoma, germ cell tumors, pineal cell tumors, chordoma, pituitary adenoma, craniopharyngioma, chordoma, acoustic schwannoma, glomus jugulare tumor, medullobastoma, hemangioblastoma, glioglastoma multiforme, neurinomas, cerebellar astrocytoma, and brainstem glioma. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with brain cancer in adults include recent onset or persistent headache, vomiting, personality and behavior changes, emotional instability, intellectual decline (*e.g.*, confusion, loss of memory, impaired calculating abilities, and impaired judgment), seizures, reduced level of consciousness, neurologic changes (*e.g.*, vision changes, hearing loss, decreased sensation of a body area, weakness of a body area, speech difficulties, and decreased coordination), fever, weakness, general ill feeling, positive Babinski's reflex, and decerebrate or decorticate posture. Common symptoms associated with brain cancer in infants include bulging fontanelles, separated sutures, opisthotonos, increased head circumference, no red reflex in the eye. Additional symptoms include tongue problems, difficulty swallowing, impaired smell, obesity, uncontrollable movement, absent menstruation, hiccups, hand tremor, facial paralysis, different pupil sizes, eyelid drooping, and breathing problem.

According to the invention, the biological composition useful for treatment of brain cancer comprises activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.501 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 8,011 MHz and a field strength of 315 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 9,147 MHz and a field strength of 312 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 9,941 MHz and a field strength of 356 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 11,335 MHz and a field strength of 332 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,032 MHz and a field strength of 375 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 11,335 MHz and a field strength of 306 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,032 MHz and a field strength of 322 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 60 μg of vitamin B_{12}, 60 μg of vitamin B_3, 60 μg of vitamin H, 50 μg of vitamin B_6, 30 ml of fetal calf serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 11,335 MHz and a field strength in the range of 300 to 355 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 318 mV/cm, 335 mV/cm, and 350 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,032 MHz and a field strength in the range of 350 to 420

mV/cm, preferably at three fields strengths, *e.g.*, in the order of 357 mV/cm, 406 mV/cm, and 415 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

Lymphoma Cancer

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from lymphoma cancer. As used herein, the term "lymphoma" includes but is not limited to Hodgkin's disease and non-Hodgkin's disease. For a detailed description of each type of lymphoma, see Rosenberg SA, Kaplan HS, eds. "Malignant Lymphomas: Etiology, Immunology, Pathology, Treatment" New York, NY, Academic Press 1982, which is incorporated herein by reference in its entirety. Classic Hodgkin's disease is divided into four subtypes: (1) nodular sclerosis Hodgkin's disease (NSHD); (2) mixed cellularity Hodgkin's disease (MCHD); (3) lymphocyte depletion Hodgkin's disease (LDHD); and (4) lymphocyte-rich classic Hodgkin's disease (cLRHD). Non-Hodgkin's lymphoma includes but is not limited to (1) slow-growing lymphoma and lymphoid leukemia (e.g., chronic lymphocytic leukemia, small lymphocytic leukemia, lymphoplasmacytoid lymphoma, follicle center lymphoma, follicular small cleaved cell, follicular mixed cell, marginal zone B-cell lymphoma, hairy cell leukemia, plasmacytoma, myeloma, large granular lymphocyte leukemia, mycosis fungoides, sézary syndrome); (2) moderately aggressive lymphomas and lymphoid leukemia (e.g., prolymphocytic leukemia, mantle cell lymphoma, follicle center lymphoma, follicular small cleaved cell, follicle center lymphoma, chronic lymphocytic leukemia/prolymphocytic leukemia, angiocentric lymphoma, angioimmunoblastic lymphoma); (3) aggressive lymphomas (e.g., large B-cell lymphoma, peripheral T-cell lymphomas, intestinal T-cell lymphoma, anaplastic large cell lymphoma); and (4) highly aggressive lymphomas and lymphoid leukemia (e.g., B-cell precursor B-lymphoblastic leukemia/lymphoma, Burkitt's lymphoma, high-grade B-cell lymphoma, Burkitt's-like T-cell precursor T-lymphoblastic leukemia/lymphoma). In a specific embodiment, the biological composition can ameliorate, reduce, manage, or eliminate the symptoms associated with lymphoma include painless swelling in one or more of the lymph nodes of the neck, collarbone region, armpits, or groin., chest pain, coughing, fatigue, shortness of

breath, fever, drenching night sweats, weight loss, fatigue, appetite loss, red patches on the skin, and severely itchy skin, often affecting the legs/feet.

According to the invention, the biological composition useful for treatment of lymphoma cancer comprises activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.562 yeast cells cultured in a series of alternating electromagnetic (EM) fields. In one preferred embodiment, the method of preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 7,977 MHz and a field strength of 282 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 7,998 MHz and a field strength of 277 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 10,029 MHz and a field strength of 387 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,837 MHz and a field strength of 442 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,862 MHz and a field strength of 325 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,837 MHz and a field strength of 391 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,862 MHz and a field strength of 315 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 60 μg of vitamin H, 40 μg of vitamin B_6, 40 μg of vitamin B_{12}, 0.20 g of KH_2PO_4, 0.20 g of $MgSO_4.7H_2O$, 0.20 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 3.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,837 MHz and a field strength in the range of 230 to 450 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 442 mV/cm, 341 mV/cm, and 244 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency at 12,862 MHz and a field strength in the range of 120 to 380 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 350 mV/cm, 224 mV/cm, and 137 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice., and soybean juice.

Leukemia

In one preferred embodiment, the invention provides a method for preparing a biological composition useful for producing a healthful benefit in a subject suffering from leukemia. As used herein, the term "leukemia" includes but is not limited to acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, and hairy cell leukemia. In a specific embodiment, the biological compositions can ameliorate, reduce, manage, or eliminate the symptoms associated with leukemia include a weakened immune system, infections, fevers, decrease in red blood cells and platelets, weakness, fatigue, loss of appetite, loss of weight, swollen or tender lymph nodes, liver, or spleen, easy bleeding or bruising, tiny red spots (called petechiae) under the skin, swollen or bleeding gums, sweating (especially at night), bone or joint pain, headaches, vomiting, confusion, loss of muscle control, and seizures.

According to the invention, the biological composition useful for treatment of leukemia comprises activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.11 yeast cells cultured in a series of alternating electromagnetic

(EM) fields. In one preferred embodiment, the method for preparing said biological composition comprises in any order the steps of:

(a) culturing the yeast cells in a first liquid medium in a first electromagnetic field having a frequency at 9,978 MHz and a field strength of 217 mV/cm;

(b) culturing the yeast cells in a first liquid medium in a second electromagnetic field having a frequency at 11,484 MHz and a field strength of 207 mV/cm;

(c) culturing the yeast cells in a first liquid medium in a third electromagnetic field having a frequency at 12,196 MHz and a field strength of 238 mV/cm;

(d) culturing the yeast cells in a first liquid medium in a fourth electromagnetic field having a frequency at 12,565 MHz and a field strength of 244 mV/cm; and

(e) culturing the yeast cells in a first liquid medium in a fifth electromagnetic field having a frequency at 12,612 MHz and a field strength of 262 mV/cm;

and after the last of the first five steps, the following steps in any order:

(f) culturing the yeast cells in a second liquid medium in a sixth electromagnetic field having a frequency at 12,565 MHz and a field strength of 265 mV/cm; and

(g) culturing the yeast cells in a second liquid medium in a seventh electromagnetic field having a frequency at 12,612 MHz and a field strength of 278 mV/cm.

Preferably, the first liquid medium comprises 20 g of sucrose or glucose, 40 μg of vitamin B_{12}, 30 μg of vitamin B_6, 20 μg of vitamin D, 20 μg of vitamin H, 30 ml of bovine calf serum, 0.25 g of $MgSO_4.7H_2O$, 0.30 g of NaCl, 0.20 g of $CaSO_4.2H_2O$, 4.0 g of $CaCO_3.5H_2O$, 2.5 g of peptone, and 1,000 ml of autoclaved water.

Preferably, the second liquid medium comprises wild hawthorn juice and gastric juice of a mammal.

The number of activated and conditioned yeast cells can be further expanded by:

(h) culturing the yeast cells in a third liquid medium in an eighth electromagnetic field or series of electromagnetic fields having a frequency at 12,565 MHz and a field strength in the range of 170 to 290 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 270 mV/cm, 277 mV/cm, and 184 mV/cm; and

(i) culturing the yeast cells in a third liquid medium in a ninth electromagnetic field or series of electromagnetic fields having a frequency 12,612 MHz and a field strength in the range of 250 to 320 mV/cm, preferably at three fields strengths, *e.g.*, in the order of 296 mV/cm, 318 mV/cm, and 267 mV/cm.

Preferably, the third liquid medium comprises wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice.

EXAMPLES: ANIMAL STUDIES

Various animal models of human cancer were used to illustrate the benefits of the biological compositions of the invention. Specifically, the effects of the biological compositions on the growth of tumors and the survival of the animals were studied.

The biological compositions used in the following animal studies comprised 10^8 per ml of activated and conditioned yeast cells that were prepared by the methods described above.

1. Lung Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *S. cerevisiae* IFFI1345 cells. The Lewis lung cancer model was used to study the growth of tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Mouse Model

The animals used to generate the Lewis lung cancer cells for the experiments were C57/B1 mice, 6 to 8 weeks old. Lewis lung carcinoma (obtainable from the National Cancer Institute, Bethesda, MD) in a suspension containing about 10^6 viable tumor cells was injected subcutaneously in six animals. The animals were allowed to grow for 21 days. The animals that showed robust growth of the tumor

were used as donors. The tumor was removed from a mouse and minced aseptically in 4 ml of Hank's solution. The suspension of tumor cells were injected into a healthy batch of C57/B1 mice that were 6 to 8 weeks old; each mouse receiving 0.2 ml of the tumor cell suspension.

The mice injected with tumor cells were divided into 3 experimental groups of ten mice per group and one control group. The three groups were triplicated (*i.e.*, using a total of 90 mice in the experimental groups). In group A, the mice received 0.3 ml of the biological composition once per day. In group B, the mice received 0.5 ml of the biological composition once per day. In group C, the mice received 0.5 ml of physiological saline once per day. A fourth group of mice, group D, which did not receive tumor cells, was given 0.3 ml of physiological saline per day.

The mice received the biological compositions or saline on the same day as the tumor cells were transplanted. The mice in group D also started receiving saline on the same day as the other three groups. The biological composition or saline were administered orally by a feeding tube for 24 consecutive days. On the 25th day from tumor inoculation, the mice were sacrificed and the weight of the lungs as well as the weight of the tumor were determined by standard techniques.

Table 7 shows the differences in the weight of the lungs and tumors of the mice in the various treatment and control groups.

Table 7

Group	**mean weight of lungs and standard deviation (mg)**	**mean weight of tumor nodules and standard deviation (mg)**
A	209 ± 12	7.2 ± 3
B	158 ± 9	2.6 ± 2
C	1056 ± 124	27.3 ± 7
D	151 ± 14	not applicable

The mice bearing Lewis cancer cells that received 0.5 ml of the biological composition of the invention (group B) showed the least deviation in the weight of lungs as compared to healthy mice not injected tumor cells (group D). The

mice of group B also had less tumor mass as compared to mice that did not receive treatment (group C) as well as the mice in group A (0.3 ml per day).

Survival Time in Mouse Model

The animals used to generate the Lewis lung cancer cells for the experiments were C57/B1 mice, 6 weeks old. Lewis lung carcinoma (obtainable from the National Cancer Institute, Bethesda, MD) in a suspension containing about 10^8 viable tumor cells was injected subcutaneously in five animals. The animals were allowed to grow for 15 days. The animals that showed robust growth of the tumor were used as donors. The tumor was removed from a mouse and minced aseptically in 4 ml of Hank's solution to form a suspension of tumor cells. 0.1 ml of the cell suspension were injected intramuscularly into each mouse. Healthy C57/B1 mice that were 6 weeks old were used.

The mice injected with tumor cells were divided into 3 experimental groups and one control group of ten mice per group. The groups were in triplicates, *i.e.*, using a total of 120 mice for the four treatments. In group A, the mice received 0.3 ml of the biological composition once per day. In group B, the mice received 0.5 ml of the biological composition once per day. In group C, the mice received 0.5 ml of physiological saline once per day. The activated and conditioned yeast cells were at a concentration of 10^8 per ml of the biological composition. A fourth group of control mice, group D, which did not receive tumor cells, was given 0.3 ml of physiological saline per day.

The mice received the biological compositions or saline 15 days after the tumor cells were transplanted. The mice in group D also started receiving saline on the same day as the other three groups. The biological composition or saline were administered orally by a feeding tube for 60 consecutive days. The mice were observed over a year from the day of tumor inoculation.

An additional set of experiments were also conducted as described above except that the mice were fed the biological composition or saline for 90 days.

Table 8 shows the number of mice in the various treatment and control group that survived the tumor injection over a period of 12 months. Each of the 30 mice in each group received 60 consecutive days of either saline or biological composition of the invention.

Table 8 Number of live animals remaining in the groups after 60 days of treatment

Time after cessation of treatment	Group A	Group B	Group C	Group D
0 month	30	29*	24	30
1 month	30	29	14	30
2 months	30	29	4	30
3 months	30	29	0	30
4 months	30	29	0	30
5 months	29*	29	0	30
6 months	29	29	0	30
7 months	29	29	0	30
8 months	29	29	0	30
9 months	29	28*	0	30
10 months	29	28	0	30
11 months	29	28	0	30
12 months	29	28	0	30

* The mice died of other causes unrelated to the lung cancer.

The above experiment was repeated with mice receiving the different treatments for 90 consecutive days instead of 60 days. Table 9 shows the number of mice in the various treatment and control group that survived the tumor injection over a period of 12 months.

Table 9 Number of live animals remaining in the groups after 90 days of treatment

Time after cessation of treatment	Group A	Group B	Group C	Group D
0 month	30	30	22	29
1 month	30	30	11	29
2 months	30	30	6	29

3 months	30	30	1	29
4 months	30	30	0	29
5 months	30	30	0	29
6 months	30	30	0	29
7 months	29*	30	0	29
8 months	29	30	0	29
9 months	29	30	0	29
10 months	29	30	0	29
11 months	29	30	0	29
12 months	29	30	0	29

* The mice died of other causes unrelated to the lung cancer.

2. Nasopharyngeal Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.116 cells. The Wistar rat model and a mouse model of human nasopharyngeal cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Wistar Rat Model

The animals used to generate the nasopharyngeal cancer cells for the experiments were Wistar rats, male and female, 190 to 210 gram in body weight. The rats were starved for 24 hours before the surgery.

Human nasopharyngeal cancer samples (obtainable from Cancer Institute, The Chinese Academy of Medical Sciences, Beijing, China) were carefully selected before, during, and after surgery in order to ensure engraftment occurred at the original position in the rat. The nasopharyngeal cancer samples were obtained from patients who have not received any radiation, chemotherapy or immune enhancement treatment. Nasopharyngeal cancer samples were divided into sections

of about 1 mm^3 in size and immediately stored in RPMI-1640 suspension after removal from the human patient.

The animals were generally anaesthetized. The barbiturates was first diluted in saline and then injected at 0.3 to 0.4 ml per animal, or 30 mg per kg body weight into the abdomen of the rats. Under sterile conditions, a 1 cm long opening in the body cavity was cut along the right rib of the rats for engraftment of the tumor. Afterwards, the opening was sutured and the rats were put back into the cage. The animals were allowed to grow for three to five weeks. The animals that showed robust growth of the tumor were used as donors. The tumor was removed from a donor rat and minced aseptically in 4 ml of Hank's solution. The suspension of tumor cells were transplanted into a healthy batch of Wistar rats for experimentation.

The rats transplanted with tumor cells were divided into 4 experimental groups of ten rats per group and one control group. The four experimental groups were triplicated (i.e., using a total of 120 rats in the experimental groups). In group AY, the rats received 2.0 ml of the biological composition once per day. In group NY, the rats received 2.0 ml of the untreated yeast cells once per day. In group TSPA, the rats were injected intravenously with 1.5 mg of thiotepa (TSPA) per kg body weight per day. In group CK1, the rats received 2.0 ml of physiological saline once per day. A fifth group of rats, group CK2, which did not receive tumor cells, was given 2.0 ml of physiological saline per day.

The rats received the biological compositions, untreated yeast cells, TSPA or saline on the same day as the tumor cells were transplanted. The rats in group CK2 also started receiving saline on the same day as the other four groups. The biological compositions, untreated yeast cells and saline were administered orally by a feeding tube and the TSPA by intravenous injection for 21 consecutive days. On the 22^{nd} day from tumor inoculation, the rats were sacrificed and the weight of the rats as well as the weight of the tumor were determined by standard techniques.

Table 10 shows the differences in the weight of the rats and tumors of the rats in the various treatment and control groups.

Table 10

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)

AY	214 ± 2.2	0.19 ± 0.21
NY	194 ± 3.2	2.3 ± 0.81
TSPA	207 ± 3.8	1.6 ± 0.74
CK1	197 ± 3.6	2.7 ± 0.84
CK2	215 ± 3.4	not applicable

The rats bearing nasopharyngeal cancer cells that received 2.0 ml of the biological composition of the invention (group AY) showed the least deviation in weight as compared to healthy rats not injected tumor cells (group CK2). The rats in group AY also had less tumor mass as compared to rats that did not receive treatment (group CK1) as well as the rats in group NY (2.0 ml of untreated yeast cells per day) and the rats in group TSPA (1.5 mg of thiotepa per kg body weight per day).

Survival Time in Wistar Rat Model

The animals were prepared in a similar manner as described immediately above. The rats transplanted with tumor cells were divided into 4 experimental groups of ten rats per group and one control group. The four experimental groups were triplicated (*i.e.*, using a total of 120 rats in the experimental groups). In group 2AY, the rats received 2.0 ml of the biological composition once per day. In group 2NY, the rats received 2.0 ml of the untreated yeast cells once per day. In group 2TSPA, the rats were injected intravenously 1.5 mg of thiotepa (TSPA) per kg body weight per day. In group 2CK1, the rats received 2.0 ml of physiological saline once per day. A fifth group of rats, group 2CK2, which did not receive tumor cells, was given 2.0 ml of physiological saline per day.

The rats received the biological compositions, untreated yeast cells, TSPA or saline on the same day as the tumor cells were transplanted. The rats in group 2CK2 also started receiving saline on the same day as the other four groups. The biological compositions, untreated yeast cells and saline were administered orally by a feeding tube and the TSPA by intravenous injection for 21 consecutive days. The rats were observed over 6 months from the day of tumor inoculation and survival was recorded. The weight of the rats and the weight of the tumor were determined by standard techniques.

Table 11 shows the number of rats in the various treatment and control group that survived the tumor injection over a period of 6 months. Each of the 30 rats in each group received 21 consecutive days of either untreated yeast cells, TSPA, saline or biological compositions of the invention. Table 6 shows the weight of the rats that survived and the weight of their tumors in the various treatment and control groups.

Table 11 Number of live animals remaining in the groups after 21 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2TSPA	Group 2CK1	Group 2CK2
0 month	30	28	30	30	30
1 month	30	24	26	22	30
2 months	30	5	24	3	30
3 months	30	0	17	0	30
4 months	30	0	7	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 12

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	226 ± 8.6	132.5 ± 10.3
2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	229 ± 11.8	not applicable

The rats bearing nasopharyngeal cancer cells that received 2.0 ml of the biological composition of the invention (group 2AY) survived for more than 6 months and the tumor never reoccurred. On the contrary, the rats in group 2NY (2.0

ml of untreated yeast cells per day), group 2TSPA (1.5 mg of thiotepa per kg body weight per day) and group 2CK1 (2.0 ml of saline per day) all died after four months from injection of tumor cells.

As in the Example immediately above (i.e. Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer), the rats bearing nasopharyngeal cancer cells that received 2.0 ml of the biological composition of the invention (group 2AY) showed the least deviation in the weight of rats as compared to healthy rats not injected tumor cells (group 2CK2).

Tumor Growth in Mouse Model

The animals used to generate the nasopharyngeal cancer cells for the experiments were nasopharyngeal cancer mice (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 6 to 8 weeks old, male and female, 18 to 20 gram in body weight. Highly differentiated nasopharyngeal cancer were obtained from the Institute of Medicine, Cancer Laboratory of China. The animals were injected with a suspension containing about 10^7 viable tumor cells subcutaneously and allowed to grow for 5 days.

The mice injected with tumor cells were divided into 4 experimental groups of ten mice per group and one control group. The four experimental groups were triplicated (i.e., using a total of 120 mice in the experimental groups). In group AY, the mice received 0.3 ml of the biological composition once per day. In group NY, the mice received 0.3 ml of the untreated yeast cells once per day. In group CTX, the mice were injected subcutaneously with 30 mg of cyclophosphamide (CTX) per kg body weight per day. In group CK1, the mice received 0.3 ml of physiological saline once per day. A fifth group of mice, group CK2, which did not receive tumor cells, was given 0.3 ml of physiological saline per day.

The mice received the biological compositions, untreated yeast cells, CTX or saline on the same day as the tumor cells were transplanted. The mice in group CK2 also started receiving saline on the same day as the other four groups. The biological compositions, untreated yeast cells and saline were administered orally by a feeding tube and the CTX by intravenous injection for 30 consecutive days. On the 31st day from tumor inoculation, the mice were sacrificed and the weight of the mice as well as the weight of the tumor were determined by standard techniques. The results are shown below.

Table 13

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	20.3 ± 1.7	0.12 ± 0.06
NY	18.5 ± 2.8	0.95 ± 0.23
CTX	19.7 ± 2.4	0.86 ± 0.13
CK1	18.1 ± 2.6	0.98 ± 0.21
CK2	20.6 ± 2.1	not applicable

Survival Time in Mouse Model

The animals were prepared in a similar manner as described immediately above. The mice injected with tumor cells were divided into 4 experimental groups of ten mice per group and one control group. The four experimental groups were triplicated (i.e., using a total of 120 mice in the experimental groups). In group 2AY, the mice received 0.3 ml of the biological composition once per day. In group 2NY, the mice received 0.3 ml of the untreated yeast cells once per day. In group 2CTX, the mice were injected intravenously with 30 mg of cyclophosphamide (CTX) per kg body weight per day. In group 2CK1, the mice received 0.3 ml of physiological saline once per day. A fifth group of mice, group 2CK2, which did not receive tumor cells were given 0.3 ml of physiological saline per day.

The mice received the biological compositions, untreated yeast cells, CTX or saline on the same day as the tumor cells were transplanted. The mice in group 2CK2 also started receiving saline on the same day as the other four groups. The biological compositions, untreated yeast cells and saline were administered orally by a feeding tube and the CTX by intravenous injection for 30 consecutive days. The mice were observed over 6 months from the day of tumor inoculation and survival was recorded. The weight of the mice and the weight of the tumor were determined by standard techniques.

Table 14 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	29	30	27	30
1 month	30	22	27	19	30
2 months	30	7	17	3	30
3 months	30	0	11	0	30
4 months	30	0	3	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 15

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.3 ± 1.6	178.5 ± 9.3
2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	20.8 ± 1.2	not applicable

3. Esophageal Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.375 cells. The Wistar rat model and a mouse model of human esophageal cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Wistar Rat Model

According to numerous animal studies N-methyl-N-benzylnitrosamine (MBNA) is a well known effective trigger of esophageal cancer in Wistar rats.

Accordingly, this experiment used MBNA in setting up a Wistar rat model of human esophageal cancer. Detailed description of the use of MBNA to induce esophageal cancer can be found in *e.g.*, Lu S.X., 1989, *Zhonghua Zhong Liu Za Zhi* 11(6):401-3; Craddock V.M. and Driver H.E., 1987, *Carcinogenesis* 8(8):1129-32, each of which is incorporated herein by reference in its entirety.

The animals used in the experiments were Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), male and female, with an average body weight of about 150 to 180 gram. The rats were given water containing 100 ppm MBNA and fed a diet containing MBNA such that each rat received 0.75 mg MBNA (obtainable from Beijing Chemical Reagent Company, China) per kg body weight per day for 30 days. All the animals essentially developed esophageal cancer.

The rats that were fed with MBNA for 30 days were divided into 4 experimental groups of ten rats per group and one control group. The four experimental groups were triplicated (*i.e.*, using a total of 120 rats in the experimental groups). In group AY, the rats received 1.5 ml of the biological composition in two doses per day. In group NY, the rats received 1.5 ml of the untreated yeast cells in two doses per day. In group VDS, the rats were injected intravenously with 3.0 mg of vindesine (VDS) per kg body weight once a week for four weeks. In group CK1, the rats received 1.5 ml of physiological saline in two doses per day. A fifth group of rats, group CK2, which did not receive tumor cells, was given 1.5 ml of physiological saline in two doses per day.

The biological compositions, untreated yeast cells and saline were administered orally by a feeding tube and the VDS by intravenous injection for 30 consecutive days. On the 31st day from tumor inoculation, the rats were sacrificed.

Table 16

Group	**mean weight of rats and standard deviation (g)**	**mean weight of esophagus and standard deviation (mg)**	**mean weight of tumor nodules and standard deviation (mg)**
AY	186 ± 14	122 ± 19	109 ± 11
NY	168 ± 17	174 ± 32	877 ± 18

VDS	173 ± 15	179 ± 27	281 ± 31
CK1	166 ± 19	176 ± 27	889 ± 26
CK2	188 ± 15	109 ± 12	not applicable

Survival Time in Wistar Rats

The experiment was carried out in a manner similar to that described immediately above, except that the survival time over a period of over 6 months was observed.

Table 17 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	**Group 2AY**	**Group 2NY**	**Group 2VDS**	**Group 2CK1**	**Group 2CK2**
0 month	30	30	30	30	30
1 month	30	27	30	30	30
2 months	30	22	30	24	30
3 months	30	17	30	19	30
4 months	30	7	21	7	30
5 months	30	0	3	0	30
6 months	30	0	0	0	30

Table 18

Group	**mean weight of rats and standard deviation (g)**	**mean weight of esophagus and standard deviation (mg)**	**mean weight of tumor nodules and standard deviation (mg)**
2AY	203 ± 22	112 ± 17	64.4 ± 0.7
2NY	all animals dead	all animals dead	all animals dead
2VDS	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	211 ± 17	101 ± 16	not applicable

Tumor Growth in TA1 Mouse Model

Numerous animal studies have reported the use of murine models in the study of human esophageal cancer. There is almost a 100% success rate for transplanting esophageal tumor SGA-73 cells in mice. Detailed description of the esophageal tumor cell line SGA-73 can be found in Ding R. *et al.*, Esophageal Cancer Experimental Research, Ren Ming Wei Shen Publisher, 1980, p. 36, which is incorporated herein by reference in its entirety.

The animals used for the experiments were TA1 mice (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 6 to 8 weeks old, male and female, with an average body weight of about 18 to 22 gram. The esophageal cancer cell line SGA-73 (obtainable from the Beijing Institute of Chinese Medicine and Pharmacology, Beijing, China) in a suspension containing about 10^7 viable tumor cells (about 0.2 ml culture suspension) was injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the Tumor Growth in Mouse Model section for the nasopharyngel cancer.

Table 19

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
AY	18.8 ± 2.7	131 ± 17
NY	17.7 ± 3.2	472 ± 23
CTX	18.2 ± 2.8	384 ± 31
CK1	17.6 ± 2.8	534 ± 25
CK2	19.4 ± 2.4	not applicable

Survival Time in Mouse Model

This experiment was carried out in a similar manner as described above in the Tumore Growth in Mouse Model for esophageal, except that the survival time of the mice was recorded for a period over 6 months. In addition, the dosages used in groups 2AY, 2NY, 2CK1 and 2CK2 were increased to 0.5 ml once per day

and the dosage for 2CTX was increased to 45 mg of cyclophosphamide (CTX) per kg body weight per day.

Table 20 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	24	30	27	30
2 months	30	16	28	21	30
3 months	30	7	22	14	30
4 months	30	2	18	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 21

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.4 ± 27.2	91.9 ± 0.7
2NY	all animals dead	all animals dead
2CTX	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.8 ± 22.2	not applicable

4. Stomach Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.14 cells. The Wistar rat model and a mouse model of human stomach cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Wistar Rat Model

Numerous animal studies have reported the use of Wistar rats in the study of treating stomach cancer. 1,2-dimethylhydrazine (DMH) is a well-known effective trigger of stomach cancer in rats. Accordingly, this experiment used DMH in designing Wistar rat model of human stomach cancer. Detailed description of the use of 1,2-dimethylhydrazine to induce stomach cancer can be found in Watanabe H. *et al.*, 1999, *Jpn J Cancer Res.* 90(11):1207-11, which is incorporated herein by reference in its entirety.

The animals used in the experiments were Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). The stomach cancer cell line (obtainable from the First Military Medical University of China, Guangzhou, China) was generated in 1991 from a Wistar rat stomach cancer which was induced by subcutaneous injection of DMH. The rats were injected subcutaneously with 2.5×10^6 stomach cancer cells per animal.

The experiment was carried out in a manner similar to that described above in the Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except that a dosage of 0.8 ml per day was used for groups AY, NY, CK1 and CK2, and a group ADM was introduced in lieu of group TSPA, wherein the rats were injected intravenously with 10^5 units of doxorubicin (adriamycin, ADM) per kg body weight per day.

Table 22

Group	**mean weight of rats and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
AY	196.6 ± 11.7	232.7 ± 9.6
NY	177.7 ± 14.5	689.6 ± 13.1
ADM	181.8 ± 12.8	446.4 ± 13.6
CK1	178.5 ± 12.5	698.5 ± 13.1
CK2	204.6 ± 13.7	not applicable

Survival Time in Wistar Rat Model

This experiment was carried out in a similar manner as described immediately above, except that survival time for a period over 6 months was observed. In addition, the dosages for groups 2AY, 2NY, 2CK1, and 2CK2 were increased to 1.2 ml per day while the dosage for 2ADM was increased to 1.5×10^5 units of doxorubicin (adriamycin, ADM) per kg body weight per day.

Table 23 Number of live animals remaining in the groups after 21 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2ADM	Group 2CK1	Group 2CK2
0 month	30	29	30	30	30
1 month	30	25	30	28	30
2 months	30	21	30	18	30
3 months	30	18	22	11	30
4 months	30	11	17	7	30
5 months	30	0	13	0	30
6 months	30	0	0	0	30

Table 24

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	213.4 ± 13.9	89.9 ± 0.7
2NY	all animals dead	all animals dead
2ADM	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	222.5 ± 11.3	not applicable

Tumor Growth in Mouse Model

Numerous animal studies have reported the use of murine models in the study of treating stomach cancer. Methylcholanthrene (MC) is a well-known effective trigger of stomach cancer in mice. Accordingly, this experiment used MC in

designing TA2 mouse model of human stomach cancer. Detailed description of the use of methylcholanthrene to induce stomach cancer can be found in Wang X.H. *et al.*, 1984, *Chin Med J (Engl)* 97(3):215-22, which is incorporated herein by reference in its entirety.

The animals used for the experiments were TA2 mice, 6 to 8 weeks old (obtainable from the Second Military Medical University, Shanghai, China). Both male and females with an average weight of about 18 to 20 gram were used. The stomach cancer cell line S784 (obtainable from MC-treated TA2 mouse provided by the Tianjian Medical University, China) in a suspension containing about 1.2×10^7 viable tumor cells (about 0.2 ml cell culture) was injected subcutaneously into the animals. The mice injected with tumor cells were kept for 5 days.

The experiment was carried in a manner similar to that described in section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group ADM was used in place of group CTX, wherein the mice were injected intravenously with 10^5 units of doxorubicin (adriamycin, ADM) per kg body weight per day.

Table 25

Group	mean weight of mice and standard deviation (g)	mean weight of stomach and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
AY	21.2 ± 0.6	412 ± 18	132 ± 9
NY	17.7 ± 0.5	778 ± 19	489 ± 31
ADM	19.8 ± 0.8	621 ± 17	346 ± 36
CK1	17.5 ± 0.5	776 ± 15	498 ± 31
CK2	21.6 ± 0.7	368 ± 17	not applicable

Survival Time in Mouse Model

This experiment is otherwise similar to that described immediately above, except that the animals were observed for a period of more than 6 months and their survival time was recorded. In addition, the dosages for groups 2AY, 2NY, 2CK1, and 2CK2 were increased to 0.5 ml per day while the dosage for 2ADM was

increased to 1.5 x 10^5 units of doxorubicin (adriamycin, ADM) per kg body weight per day.

Table 26 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2ADM	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	26	30	24	30
2 months	30	17	27	13	30
3 months	30	3	16	0	30
4 months	30	0	7	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 27

Group	mean weight of mice and standard deviation (g)	mean weight of stomach and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	21.4 ± 0.9	371 ± 12	72.2 ± 0.6
2NY	all animals dead	all animals dead	all animals dead
2ADM	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	22.5 ± 1.3	369 ± 14	not applicable

5

5. Colorectal Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.1396 cells. The Wistar rat model and a mouse model of human colorectal cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Wistar Rat Model

According to numerous animal studies, dimethyl hydrazine (DMH) is a well known effective trigger of colorectal cancer in Wistar rats. Accordingly, this experiment used DMH in setting up a Wistar rat model of human colorectal cancer. Detailed description of the use of dimethyl hydrazine to induce colorectal cancer can be found in Madarnas P. *et al.*, 1992, *Anticancer Res.* 12(1):113-7, which is incorporated herein by reference in its entirety.

A DMH mixture was prepared by mixing 400 mg DMH with 100 ml saline and 37 mg ethylene diamine tetraacetate (EDTA). The pH of the mixture was then adjusted to 6.5 using 0.1 N NaOH.

The animals used in the experiments were Wistar rats (obtained from the Chinese Academy of Military Medical Sciences, Beijing, China), male and female, with an average body weight of about 180 to 200 gram. The rats were injected subcutaneously with 21 mg of DMH mixture (as prepared above) per kg body weight once a week for 21 weeks.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except that a group AM was used in lieu of Group TSPA, wherein the rats were injected subcutaneously with 10^5 units of amycin (AM) per kg body weight per day. The animals were treated for 30 days and was sacrificed on the 31st day from tumor inoculation.

Table 28

Group	**mean weight of rats and standard deviation (g)**	**mean weight of colorectum and standard deviation (mg)**	**mean weight of tumor nodules and standard deviation (mg)**
AY	207 ± 11	1,005 ± 21	107 ± 23
NY	189 ± 15	1,564 ± 39	655 ± 47
AM	202 ± 12	1,214 ± 32	304 ± 33
CK1	187 ± 13	1,546 ± 37	626 ± 42
CK2	197 ± 18	893 ± 22	not applicable

Survival Time in Wistar Rats

The experiment was carried out in a similar manner as described immediately above, except that the rats were observed for a period of over 6 months from the day of tumor inoculation and the survival time was recorded.

Table 29 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2AM	Group 2CK1	Group 2CK2
0 month	30	30	30	28	30
1 month	30	26	30	23	30
2 months	30	11	26	17	30
3 months	30	0	22	9	30
4 months	30	0	12	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 30

Group	mean weight of rats and standard deviation (g)	mean weight of colorectum and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	226 ± 14	976 ± 17	4.2 ± 1.2
2NY	all animals dead	all animals dead	all animals dead
2AM	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	228 ± 17	917 ± 21	not applicable

Tumor Growth in Mouse Model

Numerous animal studies have reported the use of BALB/c mouse models in the study of human colorectal cancer. There is almost a 100% success rate for transplanting colorectal tumor Ca-26 cells in mice.

The animals used for the experiments were BALB/c mice (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 6 to 8 weeks old. Both males and females with average body weight of about 15 to 18 gram were used. About 10^6 viable tumor cells of the colorectal cancer cell line Ca-26 (about 0.2 ml culture suspension) were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group AM was used in lieu of Group CTX, wherein the mice were injected subcutaneously with 10^5 units of amycin (AM) per kg body weight per day.

Table 31

Group	mean weight of mice and standard deviation (g)	mean weight of colorectum and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	17.7 ± 2.0	379 ± 17	69 ± 13
2NY	17.3 ± 1.3	641 ± 29	277 ± 27
2AM	17.4 ± 1.5	587 ± 23	247 ± 29
2CK1	16.5 ± 1.7	623 ± 31	286 ± 33
2CK2	17.8 ± 2.1	326 ± 14	not applicable

Survival Time in Mouse Model

This experiment was carried out in a similar manner as described immediately above, except that the mice were observed for a period of over 6 months from the day of tumor inoculation and the survival time was recorded.

Table 32 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2AM	Group 2CK1	Group 2CK2
0 month	30	29	30	30	30
1 month	30	23	30	27	30
2 months	30	0	27	0	30

3 months	30	0	21	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 33

Group	**mean weight of rats and standard deviation (g)**	**mean weight of colorectum and standard deviation (mg)**	**mean weight of tumor nodules and standard deviation (mg)**
2AY	18.4 ± 1.4	354 ± 16	23.6 ± 0.9
2NY	all animals dead	all animals dead	all animals dead
2AM	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	18.7 ± 1.2	347 ± 19	not applicable

6. Pancreatic Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain IFFI 1413 cells. Two mouse models of human pancreatic cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Mouse Model

Numerous animal studies have reported the use of BALB/c mice in the study of treating pancreatic cancer. The use of orthotopic transplant technique has been highly successful in the development of murine models of human pancreatic cancer.

The animals used to generate the pancreatic cancer cells for the experiments were BALB/c mice, both male and female with an average body weight of about 18 to 20 gram (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). The pancreatic tumor cells were isolated from clinical

biopsy samples in 1987 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China).

The animals were starved for 24 hours before the experiment. A suspension containing about 1 x 10^6 pancreatic cancer tumor cells (about 0.2 ml culture suspension) was transplanted into the donors animals at the thorax by injection. Animals that showed robust growth of the tumor were used.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group VDS was used in lieu of Group CTX, wherein the mice were injected intravenously with 3 mg of vindesine (VDS) per kg body weight once a week for four weeks.

Table 34

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	19.8 ± 2.5	0.44 ± 0.3
NY	17.7 ± 3.2	2.86 ± 0.9
VDS	18.9 ± 3.4	1.37 ± 0.7
CK1	17.6 ± 3.6	2.84 ± 0.9
CK2	20.3 ± 2.4	not applicable

Survival Time in Mouse Model

The experiment was conducted in a similar manner as described immediately above, except that the mice were observed for a period of over 6 months from the day of tumor inoculation and the survival time was recorded.

Table 35 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VDS	Group 2CK1	Group 2CK2
0 month	30	30	30	28	30
1 month	30	27	30	22	30

2 months	30	17	27	6	30
3 months	30	0	27	0	30
4 months	29	0	11	0	30
5 months	29	0	0	0	30
6 months	29	0	0	0	30

Table 36

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
2AY	20.7 ± 3.5	78.3 ± 14.2
2NY	all animals dead	all animals dead
2VDS	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.3 ± 2.6	not applicable

Tumor Growth in Kun Ming Mouse Model

Numerous animal studies have reported the use of murine models in the study of treating pancreatic cancer. There is a 100% success rate for transplanting mice with the pancreatic tumor type MPC-83. Detailed description of the transplantable mouse pancreatic cancer cell line MPC-83 can be found in Hu M.Y., 1968, Zhonghua Zhong Liu Za Zhi (Chinese) 8(1):1-3, which is incorporated herein by reference in its entirety.

The animals used for the experiments were kun ming mice, 6 to 8 weeks old (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). Both male and females with an average weight of about 15 to 18 gram were used. The transplantable mouse pancreatic cancer cell line MPC-83 (obtainable from Kun-Ming Medical University, Kun-Ming, China) in a suspension containing about 1.2×10^7 viable tumor cells (about 0.2 ml culture suspension) was injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except

that a group MMC was used in lieu of Group CTX, wherein the mice were injected intravenously with 10^5 units of mitomycin C (MMC) per kg body weight per day.

Table 37

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	19.2 ± 2.2	0.9 ± 0.4
NY	17.4 ± 2.4	3.3 ± 1.5
MMC	18.3 ± 1.5	2.8 ± 1.2
CK1	17.6 ± 2.4	3.6 ± 1.7
CK2	20.3 ± 2.3	not applicable

Survival Time in Kun Ming Mouse Model

The experiment was carried out similar to the study described immediately above, with the exception that the mice were observed for a period of over 6 months from the day of tumor inoculation and the survival time was recorded. Moreover, the dosage was increased to 0.5 ml per day for 2AY, 2NY, 2CK1 and 2CK2 groups. For the MMC group, the dosage was increased to 1.5 x 10^5 units of mitomycin C (MMC) per kg body weight per day.

Table 38 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2MMC	Group 2CK1	Group 2CK2
0 month	30	27	30	24	30
1 month	30	0	27	0	30
2 months	30	0	21	0	30
3 months	30	0	7	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30

6 months	30	0	0	0	30

Table 39

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.5 ± 1.6	104.1 ± 16.7
2NY	all animals dead	all animals dead
2MMC	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.3 ± 2.6	not applicable

7. Liver Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.503 cells. Two mouse models of human liver cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Swiss Mouse Model

Numerous animal studies have reported the use of Swiss mice in the study of treating liver cancer. The use of orthotopic transplant technique has been highly successful in the development of mouse models of human liver cancer.

The animals used to generate the liver cancer cells for the experiments were Swiss mice about 5 weeks old (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). Both male and female Swiss mice with an average weight of about 18 to 20 gram were used in the following experiment. The mice were starved for 24 hours before the surgery. Liver tumor tissues were obtained from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China. Animal preparation was carried out in a manner similar to that described above in the nasopharyngeal cancer section.

The experimental was performed in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer.

Table 40

Group	mean weight of mice and standard deviation (g)	mean weight of liver and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
AY	21 ± 0.7	1,098 ± 21	107 ± 7
NY	18.2 ± 0.8	1,557 ± 19	579 ± 13
CTX	17 ± 0.9	1,372 ± 8	249 ± 9
CK1	19.7 ± 1.1	1,489 ± 17	601 ± 14
CK2	21 ± 0.5	997 ± 22	not applicable

Survival Time in Swiss Mouse Model

The experiment was carried out similar to the study described immediately above, except that the mice were observed for a period of over 6 months from the day of tumor inoculation and their survival time was recorded.

Table 41 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	26	30	24	30
1 month	30	22	30	16	30
2 months	30	11	27	6	30
3 months	30	0	22	0	30
4 months	30	0	19	0	30
5 months	30	0	7	0	30
6 months	30	0	0	0	30

Table 42

Group	mean weight of mice and standard deviation (g)	mean weight of liver and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	22 ± 0.6	988 ± 8	1.1 ± 0.2
2NY	all animals dead	all animals dead	all animals dead
2VDS	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	22 ± 0.8	not applicable	not applicable

Tumor Growth in C_3H Mouse Model

Numerous animal studies have reported the use of murine models in the study of treating liver cancer. Murine transplantable liver tumor type H-22 closely resembles the human liver cancer pathology. Detailed description of the murine ascites hepatoma cell line H-22 can be found in Ling M.Y., 1991, Zhonghua Zhong Liu Za Zhi 13(1):13-5, which is incorporated herein by reference in its entirety. The expression of the alphafetoprotein (AFP) gene is associated with liver cancer, and there is an association between changes in the liver tumor and the level of AFP protein in blood plasma. More information on the use of AFP in the diagnosis of liver cancer can be found in Shi X. et al., 1998, Zhonghua Zhong Liu Za Zhi 20(6):437-439, which is incorporated herein by reference in its entirety.

The animals used to generate the liver cancer cells for the experiments were C_3H mice, 6 to 8 weeks old (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). Both male and females were used. The mice were starved for 24 hours before the surgery. Liver tumor H-22 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) were injected into the mice. Ten days after transplantation, the tumors are visible and the level of AFT in plasma is detectable.

The animals were allowed to grow for 21 days. The animals that showed robust growth of the tumor were used as donors. The tumor was removed from a mouse and minced aseptically in 4 ml of Hank's solution. The suspension of tumor cells were injected into a healthy batch of C_3H mice that were 6 to 8 weeks old; each mouse receiving 0.2 ml of the tumor cell suspension.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer.

Table 43

Group	mean weight of mice and standard deviation (g)	mean weight of liver and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
AY	21.2 ± 0.6	1,142 ± 7	152 ± 6
NY	18.7 ± 0.6	1,687 ± 113	783 ± 102
CTX	18.8 ± 0.7	1,351 ± 9	253 ± 8
CK1	16.2 ± 0.8	1,774 ± 121	767 ± 121
CK2	21.6 ± 0.5	986 ± 7	not applicable

Survival Time in C_3H Mouse Model

The experiment was carried out similar to the study described immediately above, except that the mice were observed for a period of over 6 months from the day of tumor inoculation and their survival time was recorded.

Table 44 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	30	30	28	30
1 month	30	28	27	24	30
2 months	30	17	24	16	30
3 months	30	0	19	0	30
4 months	29	0	11	0	30
5 months	29	0	3	0	30
6 months	29	0	0	0	30

Table 45

Group	mean weight of mice and standard deviation (g)	mean weight of liver and standard deviation (mg)	mean weight of tumor nodules and standard deviation (mg)
2AY	23.6 ± 2.2	988 ± 16	6.1 ± 0.22
2NY	all animals dead	all animals dead	all animals dead
2VDS	all animals dead	all animals dead	all animals dead
2CK1	all animals dead	all animals dead	all animals dead
2CK2	23.7 ± 2.6	983 ± 14	not applicable

8. Testicular Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.182. The Kun Ming mouse model and the Wistar rat model of human testicular cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Kun Ming Mouse Model

Numerous animal studies have reported the use of kun ming mouse models in the study of human testicular cancer. There is almost a 100% success rate for transplanting testicular tumor HTB-104 cells in mice. The testicular tumor cell line HTB-104 can be ordered from the American Type Culture Collection.

The animals used for the experiments were kun ming mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). Only males 20 to 22 gram in body weight, 6 to 7 months old were used. About 1.2 x 10^7 viable tumor cells of the testicular cancer cell line HTB-104 (obtained from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension was injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group BLM was used in lie of the CTX group, wherein the mice were injected subcutaneously with 0.5 mg of bleomycin (BLM) per kg body weight per day.

Table 46

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	19.2 ± 2.2	0.77 ± 0.47
NY	17.7 ± 2.5	1.76 ± 0.67
BLM	18.3 ± 2.4	1.13 ± 0.65
CK1	17.6 ± 2.5	1.87 ± 0.78
CK2	19.8 ± 2.6	not applicable

Survival Time in Kun Ming Mouse Model

The experiment was carried out in a manner similar to the study described immediately above, except that the mice were observed for a period of over 6 months from the day of tumor inoculation and the survival time was recorded. In addition, the dosage was increased to 0.5 ml per day for groups 2AY, 2NY, 2CK1 and 2CK2 groups. For group 2BLM, the dosage was increased to 0.8 mg of bleomycin (BLM) per kg body weight per day.

Table 47 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2BLM	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	22	27	24	30
2 months	30	11	16	13	30
3 months	30	0	0	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 48

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	21.1 ± 2.4	89.6 ± 16.5
NY	all animals dead	all animals dead
BLM	all animals dead	all animals dead
CK1	all animals dead	all animals dead
CK2	22.6 ± 2.6	not applicable

Tumor Growth in Wistar Rat Model

Numerous animal studies have reported the use of Wistar rat models in the study of human testicular cancer. There is very high success rate for transplanting testicular tumor CRL-1973 cells in rats. The testicular tumor cell line CRL-1973 can be ordered from the American Type Culture Collection.

The animals used for the experiments were male Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China). About 2 x 10^7 viable tumor cells of the testicular cancer cell line CRL-1973 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension was injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except the dosages for groups AY, NY, CK1 and CK2 were decreased to 0.8 ml, and a group VLB was introduced in lieu of group TSPA, wherein the rats were injected intravenously with 0.4 mg of vinblastine (VLB) per kg body weight per day. Thirty (30) days of consecutive treatment was performed and the rats were sacrificed on the 31st day from tumor inoculation.

Table 49

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	193.7 ± 7.8	0.87 ± 0.34

NY	186.7 ± 8.4	3.55 ± 2.45
VLB	187.5 ± 8.5	2.67 ± 2.65
CK1	183.7 ± 8.6	3.56 ± 2.46
CK2	202.4 ± 8.6	not applicable

Survival Time in Wistar Rat Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 1.2 ml and, for group VLB, the dosage was increased to 0.55 mg of vinblastin (VLB) per kg body weight per day. The survival time for a period of over six (6) months was recorded.

Table 50 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	**Group 2AY**	**Group 2NY**	**Group 2VLB**	**Group 2CK1**	**Group 2CK2**
0 month	30	30	30	30	30
1 month	30	28	30	30	30
2 months	30	0	22	25	30
3 months	30	0	9	4	30
4 months	30	0	1	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 51

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
AY	206.5 ± 10.6	287.5 ± 32.3
NY	All animals dead	all animals dead
VLB	All animals dead	all animals dead

CK1	All animals dead	all animals dead
CK2	212.7 ± 12.5	not applicable

9. Prostate Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.440. The nude mouse model of human prostate cancer was used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Nude Mouse Model

Numerous animal studies have reported the use of nude mouse models in the study of treating prostate cancer. See, for example, People's Republic of China Pharmacopia, 2000, Chinese Health Science Publisher. The use of orthotopic transplant technique has been highly successful in the development of mouse models of human prostate cancer.

The animals used to generate the prostate cancer cells for the experiments were male nude mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China) with an average weight of about 15 to 18 gram. Fresh prostate tumor cells (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) were divided into sections of size 1-2 mm^3 and immediately stored in RPMI-1641 suspension after removal from the human patient.

The animal preparation was carried out in a manner similar to that described in the section of the nasopharyngeal cancer. The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse model for the nasopharyngeal cancer, except that a group TSPA was introduced in lieu of group CTX, wherein the mice were injected intravenously with 1.5 mg of thiotepa (TSPA) per kg body weight per day.

Table 52

Group	**mean weight of mice and**	**mean weight of tumor nodules and standard**

	standard deviation (g)	deviation (mg)
AY	17.1 ± 1.4	0.6 ± 0.7
NY	16.5 ± 1.6	3.3 ± 2.3
TSPA	16.2 ± 1.7	2.4 ± 1.2
CK1	16.3 ± 1.3	3.6 ± 2.4
CK2	17.5 ± 1.5	not applicable

Survival Time in Nude Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2TSPA, the dosage was increased to 2.2 mg of thiotepa (TSPA) per kg body weight per day. The survival time for a period of over six (6) months was recorded.

Table 53 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2TSPA	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	26	30	21	30
2 months	30	1	30	0	30
3 months	30	0	27	0	30
4 months	30	0	17	0	30
5 months	30	0	5	0	30
6 months	30	0	0	0	30

Table 54

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	18.4 ± 2.4	152.2 ± 21.4

2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	18.6 ± 2.1	not applicable

Tumor Growth in Nude Mouse Model

This experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer.

Table 55

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (g)**
AY	17.4 ± 1.4	0.7 ± 0.5
NY	16.7 ± 1.2	4.3 ± 2.2
CTX	16.7 ± 1.7	3.3 ± 1.7
CK1	16.5 ± 1.3	4.1 ± 1.8
CK2	17.6 ± 1.5	not applicable

Survival Time in Nude Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2CTX, the dosage was increased to 45 mg of cyclophosphamide (CTX) per kg body weight per day. Six (6) months of survival time was recorded.

Table 56 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	**Group 2AY**	**Group 2NY**	**Group 2CTX**	**Group 2CK1**	**Group 2CK2**
0 month	30	30	30	30	30
1 month	30	24	30	16	30

2 months	30	0	30	1	30
3 months	30	0	27	0	30
4 months	30	0	22	0	30
5 months	30	0	17	0	30
6 months	30	0	0	0	30

Table 57

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
2AY	18.8 ± 1.2	157.5 ± 11.3
2NY	All animals dead	all animals dead
2TSPA	All animals dead	all animals dead
2CK1	All animals dead	all animals dead
2CK2	19.2 ± 1.6	not applicable

10. Ovarian Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.502 cells. Two mouse models of human ovarian cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Female Mouse Model

Numerous animal studies have reported the use of orthotopic transplant technique in female mouse models to study the treating of ovarian cancer. See, for example, Animals for Experiments and Experimental Techniques, Chinese Medical Publisher, 1997. The use of orthotopic transplant technique has been highly successful in the development of mouse models of human ovarian cancer.

The animal preparation was carried out in a manner similar to that described in the section of the nasopharyngeal cancer. The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group TSPA was used in lieut of Group CTX, wherein the mice were injected intravenously with 1.5 mg of thiotepa (TSPA) per kg body weight per day.

Table 58

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	23 ± 1.6	0.7 ± 0.5
NY	20 ± 2.5	3.9 ± 1.3
TSPA	21 ± 2.1	3.1 ± 0.8
CK1	21 ± 2.2	3.7 ± 1.4
CK2	23 ± 2.7	not applicable

Survival Time in Female Mouse Model

This experiment was otherwise similar to that described immediately above, except that the animals' survival time for a period of over 6 months was recorded.

Table 59 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2TSPA	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	12	24	13	30
2 months	30	2	7	2	30
3 months	30	0	2	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30

6 months	30	0	0	0	30

Table 60

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.0 ± 4.1	36.3 ± 17
2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	20.0 ± 3.2	not applicable

Tumor Growth in C_{57}BL Mouse Model

The animals used to generate the ovarian cancer cells for the experiments were female C_{57}BL mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), six weeks old, having an average body weight of about 18 to 20 gram. Detailed description of the use of C_{57}BL mice can be found in Roby K.F. et al., 2000, Carcinogenesis 21(4):585-91, which is incorporated herein by reference in its entirety. Ovarian tumor cells (cell line obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) were injected subcutaneously into the C_{57}BL mice. The number of tumor cells injected was about 10^6 units per mouse in a 0.2 ml culture suspension.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer.

Table 61

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	21 ± 2.7	0.7 ± 0.6
NY	18 ± 2.3	4.7 ± 1.6
CTX	20 ± 2.3	3.3 ± 1.4

CK1	19 ± 2.4	4.2 ± 1.9
CK2	22 ± 2.5	not applicable

Survival Time in C_{57}BL Mouse Model

This experiment was otherwise similar to that described immediately above, except that the animals' survival time for a period of over 6 months was recorded.

Table 62 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	**Group 2AY**	**Group 2NY**	**Group 2CTX**	**Group 2CK1**	**Group 2CK2**
1 month	30	29	30	27	30
2 months	30	11	30	7	30
3 months	30	0	23	0	30
4 months	30	0	14	0	30
5 months	30	0	9	0	30
6 months	30	0	0	0	30

Table 63

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
2AY	23 ± 2.4	133.7 ± 14.7
2NY	all animals dead	all animals dead
2CTX	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	23 ± 2.9	not applicable

11. Breast Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.441 cells. Two mouse models of human breast cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Mouse Model

The murine transplantable breast cancer cell line Ca615-B was used in the model which resembles human breast cancer pathology. See e.g., In Oncology Basic and Research Protocols (Ed. J. Kao), People's Health Publishing House, Beijing, China, 1998, p.72.

The animals used to generate the breast cancer cells for the experiments were female 615 mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), having an average body weight of about 18 to 20 gram. About 10^6 viable tumor cells of the murine breast cancer cell line Ca615-B (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group TSPA was introduced in lieu of CTX group, wherein the mice were injected intravenously with 1.5 mg of thiotepa (TSPA) per kg body weight per day.

Table 64

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
AY	21 ± 2.3	0.6 ± 0.2
NY	19 ± 2.6	3.3 ± 1.5
TSPA	20 ± 2.4	3.1 ± 1.6
CK1	19 ± 2.5	3.4 ± 1.4
CK2	22 ± 2.1	not applicable

Survival Time in Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2TSPA, the dosage was increased to 2.5 mg of thiotepa (TSPA) per kg body weight per day. The survival time for a period of over six (6) months was recorded.

Table 65 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	**Group 2AY**	**Group 2NY**	**Group 2TSPA**	**Group 2CK1**	**Group 2CK2**
0 month	30	28	30	29	30
1 month	30	0	30	0	30
2 months	30	0	23	0	30
3 months	30	0	11	0	30
4 months	30	0	4	0	30
5 months	29	0	0	0	30
6 months	29	0	0	0	30

Table 66

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
2AY	22 ± 1.4	204.2 ± 19.7
2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	22 ± 2.2	not applicable

Tumor Growth in Mouse Model

Numerous studies have reported the use of mouse models in finding a treatment for breast cancer. The murine transplantable breast cancer cell line MA

782/5S-B was used in the model which resembles human breast cancer pathology. See e.g., In Oncology Basic and Research Protocols (Ed. J. Kao), People's Health Publishing House, Beijing, China, 1998, p.73.

The animals used to generate the breast cancer cells for the experiments were female mice derived from an outbreed line of mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), having an average body weight of about 18 to 20 gram. About 10^6 viable tumor cells of the breast cancer cell line MA782/5S-B (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in section Tumor Growth in Mouse Model for the nasopharyngeal cancer.

Table 67

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (g)**
AY	20.3 ± 1.9	1.7 ± 0.6
NY	19.2 ± 2.4	3.3 ± 2.4
CTX	19.4 ± 2.2	3.1 ± 1.7
CK1	19.1 ± 2.3	3.6 ± 2.2
CK2	21.2 ± 2.1	not applicable

Survival Time in Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2CTX, the dosage was increased to 45 mg of cyclophosphamide (CTX) per kg body weight per day. Six (6) months of survival time was recorded.

Table 68 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of	**Group 2AY**	**Group 2NY**	**Group 2CTX**	**Group 2CK1**	**Group 2CK2**

treatment					
0 month	30	29	30	30	30
1 month	30	16	30	11	30
2 months	30	7	17	3	30
3 months	30	0	0	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 69

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	22 ± 2.2	207.5 ± 11.3
2NY	All animals dead	all animals dead
2TSPA	All animals dead	all animals dead
2CK1	All animals dead	all animals dead
2CK2	22 ± 2.6	not applicable

12. Cervical Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.444 cells. Two mouse models of human cervical cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Mouse Model

The cervical cancer cell line U14 was used in the model which closely resembles the human cervical cancer pathology. Detailed description of the use of

cervical cancer cell line U14 can be found in Tao G. et al., 2001, Chin Med J. 114(6):623-7, which is incorporated herein by reference in its entirety.

The animals used to generate the cervical cancer cells for the experiments were female mice from the 615 strain (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China) having an average body weight of 20 to 22 gram and 4 to 6 months old. About 2 x 10^6 viable tumor cells of the cervical cancer cell line U14 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that group MMC was used in lie of group CTX, wherein the mice were injected subcutaneously with 0.8 mg of mitomycin C (MMC) per kg body weight per day.

Table 70

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
AY	20.2 ± 2.7	1.3 ± 0.32
NY	18.4 ± 3.3	4.2 ± 2.55
MMC	19.3 ± 3.3	4.4 ± 2.23
CK1	18.9 ± 3.1	4.1 ± 2.64
CK2	20.7 ± 2.2	not applicable

Survival Time in Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2MMC, the dosage was increased to 1.2 mg of mitomycin C (MMC) per kg body weight per day. Six (6) months of survival time was recorded.

Table 71 Number of live animals remaining in the groups after 30 days of treatment

Time after	**Group**	**Group**	**Group**	**Group**	**Group**

cessation of treatment	2AY	2NY	2MMC	2CK1	2CK2
0 month	30	24	30	22	30
1 month	30	4	30	2	30
2 months	30	0	24	0	30
3 months	30	0	13	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 72

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.7 ± 2.4	151.7 ± 17.4
2NY	all animals dead	all animals dead
2MMC	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.6 ± 2.3	not applicable

Tumor Growth in Kun Ming Mouse Model

The cervical cancer cell line U27 was used in the model which closely resembles the human cervical cancer pathology. Detailed description of the use of cervical cancer cell line U27 can be found in Qian S.S. et al., 1987, Zhongguo Yi Xue Ke Xue Yuan Xue Bao 9(1):33-7, which is incorporated herein by reference in its entirety.

The animals used to generate the cervical cancer cells for the experiments were female kun ming mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China) having an average body weight of 20 to 22 gram and 5 to 6 months old. About 1.2×10^7 viable tumor cells of the cervical cancer cell line U27 (obtainable from the Cancer Institute, Chinese Academy of Medical

Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that group VCR was used in lie of group CTX, wherein the mice were injected intravenously with 3 mg of vinblastine (VCR) per kg body weight per day.

Table 73

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (g)
AY	19.4 ± 2.4	1.53 ± 0.87
NY	17.9 ± 2.3	4.54 ± 2.76
VCR	18.5 ± 3.7	4.77 ± 2.79
CK1	18.3 ± 3.3	4.56 ± 2.47
CK2	20.2 ± 2.6	not applicable

Survival Time in Kun King Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2VCR, the dosage was increased to 4.5 mg of vinblastine (VCR) per kg body weight per day. Six (6) months of survival time was recorded.

Table 74 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VCR	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	17	30	27	30
2 months	30	4	22	11	30
3 months	30	0	17	0	30
4 months	30	0	4	0	30

5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 75

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	21.4 ± 2.6	98.7 ± 16.6
2NY	all animals dead	all animals dead
2VCR	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	22.2 ± 2.5	not applicable

13. Uterine Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.605 cells. Two mouse models of human uterine cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Mouse Model

Murine transplantable uterine cancer type CRL-1622 closely resembles the human uterine cancer pathology. The uterine cancer cell line CRL-1622 can be ordered from the American Type Culture Collection.

The animals used to generate the uterine cancer cells for the experiments were female 615 mice (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 4 to 6 months, having an average body weight of about 20 to 22 gram. About 1.2 x 10^7 viable tumor cells of the uterine cancer cell line CRL-1622 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that group MMC was used in lie of group CTX, wherein the mice were injected subcutaneously with 0.8 mg of mitomycin C (MMC) per kg body weight per day.

Table 76

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
AY	18.9 ± 1.7	1.56 ± 0.54
NY	17.6 ± 2.3	4.43 ± 2.45
MMC	18.3 ± 3.1	3.56 ± 2.32
CK1	17.4 ± 3.7	4.82 ± 2.46
CK2	19.6 ± 2.6	not applicable

Survival Time in Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2MMC, the dosage was increased to 1.2 mg of mitomycin C (MMC) per kg body weight per day. Six (6) months of survival time was recorded.

Table 77 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2MMC	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	21	28	23	30
2 months	30	11	26	12	30
3 months	30	2	18	3	30
4 months	30	0	7	0	30
5 months	30	0	0	0	30

6 months	30	0	0	0	30

Table 78

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.2 ± 2.5	78.7 ± 17.4
2NY	all animals dead	all animals dead
2MMC	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.4 ± 2.6	not applicable

Tumor Growth in Kun Ming Mouse Model

The animals used to generate the uterine cancer cells for the experiments were female kun ming mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 5 to 6 months, having an average body weight of about 20 to 22 gram. About 1.2 x 10^7 viable tumor cells of the uterine cancer cell line HTB-114 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that group VCR was used in lie of group CTX, wherein the mice were injected intravenously with 3 mg of vinblastine (VCR) per kg body weight per day.

Table 79

Group	mean weight of mice and standard deviation (g)	Mean weight of tumor nodules and standard deviation (g)
AY	19.6 ± 2.6	1.65 ± 0.67
NY	17.7 ± 2.7	4.33 ± 2.34
VCR	18.3 ± 3.3	4.36 ± 2.45

CK1	18.4 ± 3.4	4.32 ± 2.36
CK2	20.5 ± 2.5	not applicable

Survival Time in Kun Ming Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2VCR, the dosage was increased to 4.5 mg of vinblastine (VCR) per kg body weight per day. Six (6) months of survival time was recorded.

Table 80 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	**Group 2AY**	**Group 2NY**	**Group 2VCR**	**Group 2CK1**	**Group 2CK2**
0 month	30	30	30	30	30
1 month	30	30	28	21	30
2 months	30	16	19	17	30
3 months	30	3	11	5	30
4 months	30	0	3	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 81

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
2AY	21.6 ± 2.7	103.5 ± 17.8
2NY	all animals dead	all animals dead
2VCR	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	22.7 ± 2.4	not applicable

14. Kidney Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces carlsbergensis* Hansen strain AS2.189 cells. A hamster model and the Wistar rat model of human kidney cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Hamster Model

The following example illustrates the benefit of a biological composition of the invention in a Syrian hamster model of human kidney cancer. The growth of the tumor in the hamsters was studied. Detailed description of the use of Syrian hamster to study kidney cancer can be found in, e.g., Liehr et al., 1991, Carcinogenesis 12:385-9, which is incorporated herein by reference in its entirety.

Complex cancer cells of kidney closely resembles the human kidney cancer pathology. See e.g., Modern Clinical Experimental Protocols, People's Republic of China Health Publishing House, 1997.

The animals used to generate the kidney cancer cells for the experiments were Syrian hamsters (obtainable from the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences, Beijing, China), an equal number of male and female, 5 to 6 months old, having an average body weight of 150 to 180 gram. The complex cancer cells of kidney was generated from neutered male Syrian hamsters which were induced by subcutaneous injection of 17-β-hydroxysteroid. About 2 x 10^6 viable tumor cells of the complex cancer cells of kidney (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that of the section Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that the dosages for groups AY, NY, CK1 and CK2 were 0.5 ml once per. In addition, group FUDR was used in lieu of group CTX, wherein the hamsters were injected subcutaneously with 0.6 mg of fluoruridine (FUDR) per kg body weight per day.

Table 82

Group	mean weight of hamsters and standard deviation (g)	Mean weight of tumor nodules and standard deviation (mg)
AY	122.5 ± 6.4	0.6 ± 0.4
NY	108.4 ± 4.4	3.3 ± 1.7
FUDR	116.7 ± 4.9	2.7 ± 1.2
CK1	106.6 ± 4.7	3.2 ± 1.8
CK2	123.7 ± 3.8	Not applicable

Survival Time in Hamster Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 83 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2FUDR	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	25	27	21	30
2 months	30	9	11	7	30
3 months	30	0	5	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 84

Group	mean weight of hamsters and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)

2AY	134.8 ± 5.6	187.3 ± 12.6
2NY	all animals dead	all animals dead
2FUDR	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	142.7 ± 7.9	not applicable

Tumor Growth in Wistar Rat Model

Complex cancer cells of kidney closely resembles the human kidney cancer pathology. See e.g., Modern Clinical Experimental Protocols, People's Republic of China Health Publishing House, 1997.

The animals used to generate the kidney cancer cells for the experiments were Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China) having an average body weight of about 180 to 200 gram. The complex cancer cells of kidney (obtainable from the Shanghai Medical University, Shanghai, China) were generated from a Wistar rat with kidney cancer that was induced by subcutaneous injection of bismaleimidohexane (BHM). About 2.5×10^7 viable tumor cells of the complex cancer cells of kidney in about 0.4 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that of the section Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except that the dosages for groups AY, NY, CK1 and CK2 were 0.8 ml once per, and a group VCR was used in lieu of group TSPA, wherein the rats were injected intravenously with 3 mg of vinblastine (VCR) per kg body weight per day. The treatment continued for 30 days and the rats were scarified on the 31st day.

Table 85

Group	**mean weight of rats and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (g)**
AY	197.4 ± 12.2	0.6 ± 0.4
NY	185.2 ± 12.7	4.4 ± 2.4
VCR	189.6 ± 2.5	3.7 ± 1.9

CK1	184.4 ± 12.9	4.3 ± 2.6
CK2	198.6 ± 12.3	not applicable

Survival Time in Wistar Rat Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 86 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	**Group 2AY**	**Group 2NY**	**Group 2VCR**	**Group 2CK1**	**Group 2CK2**
0 month	30	28	30	30	30
1 month	30	22	30	26	30
2 months	30	19	30	16	30
3 months	30	7	22	4	30
4 months	30	3	13	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 87

Group	**mean weight of rats and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
2AY	216 ± 12.4	257.5 ± 12.6
2NY	All animals dead	all animals dead
2VCR	All animals dead	all animals dead
2CK1	All animals dead	all animals dead
2CK2	221 ± 12.1	not applicable

15. Bladder Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.4 cells. Mouse model and Wistar rat model of bladder cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Mouse Model

Numerous animal studies have reported the use of murine transplantable bladder cancer animal models. Murine transplantable bladder cancer cell type N-butyl-N-(4-hydroxybutylnitrosamine (BBN) induced T_{739} closely resembles the human bladder cancer pathology. Detailed description of the use of murine transplantable bladder cancer cell type BBN-induced T_{739} can be found in, e.g., Li L. et al., 2002, Sheng Wu Hua Xue Yu Sheng 34:21-7, which is incorporated herein by reference in its entirety.

The BBN-induced T_{739} cells (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) were obtained from a 12^{th} generation cell line.

The animals used to receive the bladder cancer cells were T739 mouse (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China) having an average body weight of about 18 to 20 gram. About 10^6 viable tumor cells of the bladder cancer cells T_{739} (in about 0.2 ml culture suspension) were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that group TSPA was used in lieu of group CTX, wherein the mice were injected intravenously with 1.5 mg of thiotepa (TSPA) per kg body weight per day.

Table 88

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
AY	20.8 ± 2.9	1.3 ± 0.6*
NY	18.2 ± 2.1	4.2 ± 2.7

TSPA	19.5 ± 2.2	3.3 ± 2.1
CK1	18.4 ± 2.7	3.9 ± 2.4
CK2	21.3 ± 2.2	not applicable

Survival Time in Mouse Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 89 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	**Group 2AY**	**Group 2NY**	**Group 2TSPA**	**Group 2CK1**	**Group 2CK2**
0 month	30	26	30	24	30
1 month	30	19	24	21	30
2 months	30	8	11	7	30
3 months	30	0	7	0	30
4 months	30	0	2	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 90

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
2AY	22.7 ± 2.7	143.3 ± 12
2NY	all animals dead	all animals dead
2TSPA	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	22.5 ± 2.4	not applicable

Tumor Growth in Wistar Rat Model

Murine transplantable bladder cancer type N-butyl-N-(4-hydroxybutylnitrosamine (BBN) closely resembles the human bladder cancer pathology. Numerous animal studies have reported the use of murine transplantable bladder cancer animal models. Detailed description of the use of murine transplantable bladder cancer cell type N-butyl-N-(4-hydroxybutyl)nitrosamine can be found in, e.g., Lummen G. et al., 2002, Urol Res. 30:199-203, which is incorporated herein by reference in its entirety.

The animals used to generate the bladder cancer cells for the experiments are Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), 8 weeks old, with an average body weight of about 180 to 200 gram. Under sterile conditions, about 10^6 viable tumor cells of the bladder cell line BBN (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that of the section Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except that the dosages for groups AY, NY, CK1 and CK2 were 0.3 ml once per, and a group VCR was used in lieu of group TSPA, wherein the rats were injected intravenously with 3 mg of vinblastine (VCR) per kg body weight per day. The treatment continued for 30 days and the rats were scarified on the 31st day.

Table 91

Group	**mean weight of rats and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (g)**
AY	203.4 ± 6.3	0.73 ± 0.12*
NY	187.5 ± 13.1	3.44 ± 2.6
VCR	191.3 ± 12.4	2.65 ± 1.7
CK1	182.8 ± 12.8	3.36 ± 2.4
CK2	204.2 ± 12.4	not applicable

Survival Time in Wistar Rat Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 92 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VCR	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	30	30	30	30
2 months	30	24	30	22	30
3 months	30	13	30	17	30
4 months	30	0	30	8	30
5 months	30	0	28	0	30
6 months	30	0	26	0	30

Table 93

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	218 ± 12.2	37.5 ± 9.7
2NY	all animals dead	all animals dead
2VCR	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	209 ± 11.8	not applicable

16. Brain Cancer

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.501 cells. The Kun Ming mouse model and Wistar rat

model of human brain cancer were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Tumor Growth in Kun Ming Mouse Model

The kun ming murine brain cancer cell line B22 was used in the model which resembles human brain cancer pathology. See e.g., In Oncology Basic and Research Protocols (Ed. J. Kao), People's Health Publishing House, Beijing, China, 1998, p.78.

The animals used to generate the brain cancer cells for the experiments are male kun ming mice (obtainable from the Chinese Academy of Military Medicine Sciences, Beijing, China), 6 to 7 months, having an average body weight of about 20 to 22 gram. About 1.2×10^7 viable tumor cells of the kun ming murine brain cancer cell line B22 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China) in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that a group BCNU was used in lieu of group CTX, wherein the mice were injected subcutaneously with 0.8 mg of chloroethylnitrosourea (BCNU) per kg body weight per day.

Table 94

Group	**mean weight of mice and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (mg)**
AY	18.7 ± 1.9	0.65 ± 0.45
NY	17.5 ± 2.6	1.56 ± 0.54
BCNU	18.4 ± 3.4	1.02 ± 0.52
CK1	17.6 ± 3.4	1.82 ± 0.65
CK2	19.5 ± 2.4	not applicable

Survival Time in Kun Ming Mouse Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 0.5 ml and, for group 2BCNU, the dosage was increased to 1.2 mg of chloroethylnitrosourea (BCNU) per kg body weight per day. Six (6) months of survival time was recorded.

Table 95 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2BCNU	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	17	21	23	30
2 months	30	7	15	8	30
3 months	30	0	4	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 96

Group	mean weight of mice and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	20.6 ± 2.6	88.6 ± 16.7
2NY	all animals dead	all animals dead
2BCNU	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	21.5 ± 2.7	not applicable

Tumor Growth in Wistar Rat Model

The Wistar King rat brain cancer cell line WKS-1 was used in the model which resembles human brain cancer pathology. See e.g., In Oncology Basic

and Research Protocols (Ed. J. Kao), People's Health Publishing House, Beijing, China, 1998, p.79.

The animals used to generate the brain cancer cells for the experiments are Wistar rats (obtainable from the Chinese Academy of Military Medical Sciences, Beijing, China), both males and females, having an average body weight of about 180 to 200 gram. About 2 x 10^7 viable tumor cells of the Wistar King rat brain cancer type WKS-1 (obtainable from the Shanghai Medical University, Shanghai, China) in about 0.4 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that in the section of Tumor Growth in Wistar Rat Model for the nasopharyngeal cancer, except that the dosages for groups AY, NY, CK1 and CK2 were 0.8 ml once per, and a group VCR was used in lieu of group TSPA, wherein the rats were injected intravenously with 3 mg of vinblastine (VCR) per kg body weight per day. The treatment continued for 30 days and the rats were scarified on the 31st day.

Table 97

Group	**mean weight of rats and standard deviation (g)**	**mean weight of tumor nodules and standard deviation (g)**
AY	196.6 ± 9.6	0.76 ± 0.38
NY	187.7 ± 8.7	3.83 ± 2.43
VCR	188.3 ± 8.3	2.87 ± 2.54
CK1	184.4 ± 8.4	3.92 ± 2.66
CK2	201.5 ± 8.5	not applicable

Survival Time in Wistar Rat Model

This experiment was otherwise similar to that described immediately above, except that, for groups 2AY, 2NY, 2CK1, and 2CK2, the dosages were increased to 1.2 ml and, for group 2VCR, the dosage was increased to 4.5 mg of vinblastine (VCR) per kg body weight per day. Six (6) months of survival time was recorded.

Table 98 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2VCR	Group 2CK1	Group 2CK2
0 month	30	30	30	30	30
1 month	30	30	30	30	30
2 months	30	22	30	30	30
3 months	30	14	17	19	30
4 months	30	4	7	6	30
5 months	30	0	3	0	30
6 months	30	0	0	0	30

Table 99

Group	mean weight of rats and standard deviation (g)	mean weight of tumor nodules and standard deviation (mg)
2AY	212.6 ± 12.7	313.5 ± 24.6
2NY	all animals dead	all animals dead
2VCR	all animals dead	all animals dead
2CK1	all animals dead	all animals dead
2CK2	223.7 ± 12.4	not applicable

17. Lymphoma

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces cerevisiae* Hansen strain AS2.562 cells. Two mouse models of lymphoma were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment.

Inbred PBA Mouse Model

A model based on inbred PBA mice which manifest a high incidence of tumor was used (Bailey et al., 1970, J Natl. Cancer Inst. 45(1):59-73). Mice six to

eight weeks old were treated by the method as described in Shiyan Dongwu Yu Zhongliu Yanjiu (Experimental Animal & Tumor Research), April 2000, by Chinese Medical Technology Publisher.

The mice that developed lymphoma spontaneously and that had been treated by the method as described above were divided into two series of five groups: one series received treatment for 30 days, the other series received treatment for 60 days. In any one experimental series, each treatment group has a total of 36 mice.

In the first series, the mice in group AY received 0.3 ml of the oral composition of the invention per day. The mice in group NY received 0.3 ml per day of a composition comprising at a similar concentration the same strain of yeast which had not been activated and conditioned. The mice in group CTX received subcutaneous injection of cyclophosphamide at a dose of 30 mg per kg body weight per day. The mice in group CK1 received physiological saline, while the mice in group CK2 received physiological saline but did not receive transplanted lymphoma cells. The experiment was carried out over a 30-day treatment period.

In the second series, the mice in group 2AY received 0.5 ml of the oral composition of the invention per day. The mice in group 2NY received 0.5 ml per day of a composition comprising the same strain of yeast which had not been activated and conditioned at a similar concentration. The mice in group 2CTX received subcutaneous injection of cyclophosphamide at a dose of 45 mg per kg body weight per day. The mice in group 2CK1 received physiological saline, while the mice in group 2CK2 received physiological saline but did not receive lymphoma cells. The experiment was carried out over a 60-day treatment period.

After the 30-day or 60-day treatment, the mice were fed a normal diet and allowed to live under the same environment. The health and survival of the mice were observed and compared over 180 days.

The results of the first series of five groups which received treatment for a total of 30 days are shown in Table 4. Each group consisted of 36 mice at the beginning of the experiment (3 repeats). The total number of surviving mice in each group were counted at the end of every month up to 6 months after treatment stopped.

Table 100

Days after treatment period	Group CK2 (saline, no lymphoma cells)	Group CK1 (saline only)	Group CTX (30 mg/kg cyclophosphamide ml per day)	Group AY (0.3 ml oral compositions per day)	Group NY (0.3 ml non-activated yeasts per day)
0	36	34	36	36	33
30	36	22	33	36	26
60	36	0	27	36	21
90	36	0	19	36	0
120	36	0	17	34	0
150	36	0	0	34	0
180	36	0	0	34	0

The results of the second series of five groups which received treatment for a total of 60 days are shown in Table 5. Each group consisted of 36 mice at the beginning of the experiment (3 repeats). The total number of surviving mice in each group were counted at the end of every month up to 6 months after treatment stopped.

Table 101

Days after treatment period	Group 2CK2 (saline, no lymphoma cells)	Group 2CK1 (saline only)	Group 2CTX (30 mg/kg cyclophosphamide ml per day)	Group 2AY (0.5 ml oral compositions per day)	Group 2NY (0.5 ml non-activated yeasts per day)
0	36	36	36	36	35
30	36	25	36	36	28
60	36	11	29	36	19
90	36	4	21	36	11
120	36	0	14	36	6
150	36	0	14	36	0

180	36	0	8	36	0

The above results indicate that the lifespan of PBA mice with spontaneous lymphoma was prolonged as a result of receiving the oral composition of the invention at a dose of 0.3 ml per day or 0.5 ml per day for a 30-day or 60-day treatment period. The results also showed that in comparison to cyclophosphamide, the oral composition of the invention at a dose of 0.3 ml per day or 0.5 ml per day for a 30-day or 60-day treatment period prevented the recurrence of the lymphoma in the animals after treatment ceased.

Athymic Nude Mouse Model

A model based on transplantation of Burkitt's lymphoma cell in athymic nude mice was used (Gurtsevitch et al., 1988, Int J Cancer 15;41(1):87-95). The model was set up as described in Shiyan Dongwu Yu Zhongliu Yanjiu (Experimental Animal & Tumor Research), April 2000, by Chinese Medical Technology Publisher. Essentially, seven week old mice were injected intravenously each with 1×10^6 Burkitt's lymphoma cells.

Twenty-one days after the injection of lymphoma cells, the mice were divided into two series of five groups: one series received treatment for 30 days, the other series received treatment for 60 days. In any one experimental series, each treatment group has a total of 36 mice. The mice in group A received saline orally. The mice in group B received subcutaneous injection of cyclophosphamide at a dose of 30 mg per kg body weight per day. Group C received subcutaneous injection of cyclophosphamide at a dose of 45 mg per kg body weight per day. The mice in group D received 0.3 ml of the oral composition per day. group E received 0.5 ml of the oral composition per day.

After the 30-day or 60-day treatment, the mice were fed a normal diet and allowed to live under the same environment. The health and survival of the nude mice were observed and compared over 360 days.

The results of the first series of five groups which received treatment for a total of 30 days are shown in Table 7. Each group consisted of 36 mice at the beginning of the experiment (3 repeats). The total number of surviving mice in each group were counted at the end of every month up to 12 months after treatment stopped.

Table 102

Days after treatment period	Group A (0.5 saline per day)	Group B (30 mg/kg cyclophosphamide ml per day)	Group C (45 mg/kg cyclophosphamide ml per day)	Group D (0.3 ml oral compositions per day)	Group E (0.5 ml oral compositions per day)
0	36	36	35	36	36
30	36	33	35	35*	36
60	36	24	27	35	36
90	35*	14	21	35	36
120	35	5	17	35	36
150	35	3	11	35	36
180	35	1	4	35	36
210	35	0	2	35	36
240	35	0	1	35	36
270	35	0	0	35	34*
300	34*	0	0	35	34
330	34	0	0	35	34
360	34	0	0	35	34

* The mice died of other causes unrelated to the lymphoma.

The results of the second series of five groups which received treatment for a total of 60 days are shown in Table 6. Each group consisted of 36 mice at the beginning of the experiment (3 repeats). The total number of surviving mice in each group were counted at the end of every month up to 12 months after treatment stopped.

Table 103

Days after treatment period	Group A (0.5 saline per day)	Group B (30 mg/kg cyclophosphamide ml per day)	Group C (45 mg/kg cyclophosphamide ml per day)	Group D (0.3 ml oral compositions per day)	Group E (0.5 ml oral compositions per day)
0	36	35	34	35*	36
30	36	35	34	35	36
60	36	33	34	35	36
90	36	24	27	35	36
120	36	19	19	35	36
150	36	9	12	35	36
180	36	5	7	35	36
210	36	2	3	35	36
240	36	0	0	35	36
270	36	0	0	35	35*
300	36	0	0	35	35
330	34*	0	0	35	35
360	34	0	0	35	35

* The mice died of other causes unrelated to the lymphoma.

The above results indicate that the lifespan of athymic nude mice transplanted with Burkitt's lymphoma cells was prolonged as a result of receiving the oral composition of the invention at a dose of 0.3 ml per day or 0.5 ml per day for a 30-day or 60-day treatment period. The results also showed that in comparison to cyclophosphamide, the oral composition of the invention at a dose of 0.3 ml per day or 0.5 ml per day for a 30-day or 60-day treatment period prevented the recurrence of the lymphoma in the animals after treatment ceased.

18. Leukemia

The following example illustrates the benefit of a biological composition of the invention comprising activated and conditioned *Saccharomyces*

cerevisiae Hansen strain AS2.11 cells. Two mouse models of human leukemia were used to study the growth of the tumors in animals and the survival time thereof after tumor injection and treatment. Leukemia cell line HL-60 was also used to study the benefit of the biological composition in regulating the changes in cell cycles.

Tumor Growth in SWI Mouse Model

Murine transplantable leukemia cell line L6565 closely resembles the human leukemia pathology. See, *e.g.*, Lab Animal and Animal Experiment Techniques, China Chinese Medicine Publisher, 1997, which is incorporated herein by reference in its entirety.

The animals used to generate the leukemia cells for the experiments were SWI mice (obtainable from the Chinese Academy of Military Medicine Science, Beijing, China), both males and females, with an average body weight of about 18 to 22 gram. About 10^6 viable tumor cells of the leukemia cell line L6565 (obtainable from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, Cancer) in about 0.2 ml spleen cell culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that 21 days of consecutive treatment was performed in lieu of the 30 day treatment regime.

Table 104

Group	number of leukemia cells and standard deviation (cells/ml)
AY	0.0 ± 0.0
NY	2,930 ± 798 (all animals died between 9 to 13 days after injection of tumor; the number of tumor cells were measured at the time of death)
CTX	933 ± 241
CK1	2,860 ± 831 (all animals died between 9 to 15 days after injection of tumor; the number of tumor cells were measured at the time of death)
CK2	0.0 ± 0.0

Survival Time in SWI Mouse Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 105 Number of live animals remaining in the groups after 21 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	19	28	17	30
1 month	30	0	19	0	30
2 months	30	0	3	0	30
3 months	30	0	0	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30
6 months	30	0	0	0	30

Table 106

Group	number of leukemia cells and standard deviation (cells/ml)
2AY	0.0 ± 0.0
2NY	all animals dead
2CTX	all animals dead
2CK1	all animals dead
2CK2	0.0 ± 0.0

Tumor Growth in T_{739} Mouse Model

Detailed description of the use of T_{739} mice to study cancer can be found in, *e.g.*, Niu Q. *et al.*, 2001, *Zhonghua Zhong Liu Za Zhi.* 23(5):382-4, and Zheng F. *et al.*, 2000, *Zhonghua Jie He He Hu Xi Za Zhi.* 23(1):34-6, each of which is incorporated herein by reference in its entirety.

The animals used to generate the leukemia cells for the experiments were T_{739} mouse (obtainable from the Chinese Academy of Military Medicine Sciences, Beijing, China), both males and females, with an average body weight of

about 18 to 22 gram. About 10^6 viable tumor cells of the leukemia cell line T_{739} in about 0.2 ml culture suspension were injected subcutaneously into the animals.

The experiment was carried out in a manner similar to that described in the section of Tumor Growth in Mouse Model for the nasopharyngeal cancer, except that 21 days of consecutive treatment was performed in lieu of the 30 day treatment regime.

Table 107

Group	number of leukemia cells and standard deviation (cells/ml)
AY	0.0 ± 0.0
NY	2,032 ± 643 (all animals died between 10 to 15 days after injection of tumor; the number of tumor cells were measured at the time of death)
CTX	957 ± 132
CK1	2,087 ± 712 (all animals died between 11 to 17 days after injection of tumor; the number of tumor cells were measured at the time of death)
CK2	0.0 ± 0.0

Survival Time in T_{739} Mouse Model

This experiment was otherwise similar to that described immediately above, except that the animals were observed for over six (6) months and their survival time was recorded.

Table 108 Number of live animals remaining in the groups after 30 days of treatment

Time after cessation of treatment	Group 2AY	Group 2NY	Group 2CTX	Group 2CK1	Group 2CK2
0 month	30	0	28	0	30
1 month	30	0	19	0	30
2 months	30	0	3	0	30
3 months	30	0	0	0	30
4 months	30	0	0	0	30
5 months	30	0	0	0	30

6 months	30	0	0	0	30

Table 109

Group	number of leukemia cells and standard deviation (cells/ml)
2AY	0.0 ± 0.0
2NY	all animals dead
2CTX	all animals dead
2CK1	all animals dead
2CK2	0.0 ± 0.0

Cell Regulation

The following example illustrates the benefit of a biological composition of the invention in regulating cell changes in a leukemia cell line HL-60. The leukemia cell line HL-60 is commonly used to establish the efficacy of a treatment. See, *e.g.*, Modern Pharmacology Experimental Protocols, Peking Union Medical College Publisher, 1998.

Flow cytometry was used to study the changes in cell cycles. Flow cytometry measures the number of cells in different cell cycles by analyzing the amount of DNA in a single cell. A fluorescent dye, propidium iodide (PI) intercalates with DNA and emits yellow and red regions fluorescence in response to 488 nm excitation. Cells in the G1 and G0 cell cycle phases will contain a 2n amount of DNA. Cells in the G2 and M phases will contain a 4n amount of DNA, while cells in S phase will have a DNA content between 2n and 4n (as the DNA has only partially been replicated). In particular, flow cytometry can be used to determine the percentage of live cells in each phase of the cell cycle and the percentage of dead cells. This information, coupled with the doubling time, allows one to calculate the length of each cell cycle phase. More detailed description of the methodologies and applications of flow cytometry can be found in Darzynkiewicz *et al.*, 2001, *Clin Lab Med.* 21(4):857-73, which is incorporated by reference herein in its entirety.

Activated HL-60 leukemia cells (obtainable from the Cancer Institute, Chinese Academy of Military Medical Sciences, Beijing, China) were cultured in RPMI-1640 suspension and then divided into 3 groups of 10^9 cells per group. The

three groups of HL-60 cells were quintuplicated. Detailed description of the cultivation of human leukemia HL-60 cells can be found in, *e.g.*, Saether A.K. *et al.*, 1991, *Eur J Cell Biol.* 55(2):346-51, which is incorporated by reference herein in its entirety.

In the experimental group AY, the biological composition was filtered through 0.22 μm filter. The activated and conditioned yeast cells (about 5 μm in width and 10 μm in length) were collected and added to 10^9 HL-60 leukemia cells per ml to make 5% final concentration by weight. In the comparative group NY, the untreated yeast cells were filtered through 0.22 μm filter. The untreated yeast cells (about 5 μm in width and 10 μm in length) were collected and added to 10^9 HL-60 leukemia cells per ml to make 5% final concentration by weight. In the control group CK, 10^9 HL-60 leukemia cells per ml was used.

Each group of cells were observed after culturing for 24 hours and 72 hours. Afterwards, the cells were stained with propidium iodide and the percentage of cells at different cell cycle stages were observed using flow cytometry. Detailed description of the use of flow cytometry to study different cell cycle stages can be found in Hagisawa S. et al., *Med Electron Microsc.* 1999;32(3):167-174).

Tables 10 and 11 show the percentages of live leukemia cells in each cell cycle and the percentages of dead leukemia cells in the treatment and control groups after culturing for 24 hours and 72 hours, respectively.

Table 110 Percentages of live leukemia cells and dead leukemia cells after 24 hours

Cell Cycle Stage	AY (% of cells)	NY (% of cells)	CK (% of cells)
G_0-G_1	28.9%	33.2%	33.0%
S	50.8%	44.4%	44.3%
G_2-M	20.3%	22.4%	22.6%
Programmed Cell Death	24.7%	3.2%	3.1%

After culturing the HL-60 leukemia cells with the activated and conditioned yeast cells for 24 hours (group AY), 24.7% of the HL-60 leukemia cells were dead and 75.3% of the leukemia cells were alive (100% - 24.7%). Out of the total number of live leukemia cells, 28.9% were in G_0-G_1 stage; 50.8% were in S

stage; and 20.3% were in G_2-M stage, *i.e.*, 28.9% + 50.8% + 20.3% = 100% (total live leukemia cells).

In contrast, after culturing the HL-60 leukemia cells with untreated yeast cells for 24 hours (group NY), 3.2% of the leukemia cells were dead and 96.8% of the leukemia cells were alive (100% - 3.2%). Out of the total number of live leukemia cells, 33.2% were in G_0-G_1 stage; 44.4% were in S stage; and 22.4% were in G_2-M stage, *i.e.*, 33.2% + 44.4% + 22.4% = 100% (total live leukemia cells).

Similarly, after culturing the HL-60 leukemia cells alone for 24 hours (group CK), only 3.1% of the leukemia cells were dead and 96.9% of the leukemia cells were alive (100% - 3.1%). Out of the total number of live leukemia cells, 33.0% were in G_0-G_1 stage; 44.3% were in S stage; and 22.6% were in G_2-M stage, *i.e.*, 33.0% + 44.3% + 22.6% = 99.9% (total live leukemia cells).

After 24 hours, the percentage of programmed cell death is significantly greater (8x) when the HL-60 leukemia cells were co-incubated with the activated and conditioned yeast cells of the present invention (24.7% in group AY) than when the HL-60 leukemia cells were co-incubated with the untreated yeast cells (3.2% in group NY) or cultured alone (3.1% in group CK). At 24 hours, co-incubation with the activated and conditioned yeast cells of the present invention have already begun to reduce the number of live leukemia cells. A significant number of live leukemia cells in all three groups were at S stage, *i.e.*, when DNA replication occurs (synthesis phase).

Table 111 Percentages of live leukemia cells and dead leukemia cells after 72 hours

Cell Cycle Stage	**AY (% of cells)**	**NY (% of cells)**	**CK (% of cells)**
G_0-G_1	56.2%	41.6%	41.3%
S	36.1%	40.2%	40.7%
G_2-M	7.7%	18.2%	18.0%
Programmed Cell Death	85.2%	11.6%	11.3%

After culturing the HL-60 leukemia cells with the activated and conditioned yeast cells for 72 hours (group AY), 85.2% of the leukemia cells were dead and 14.8% of the leukemia cells were alive (100% - 85.2%). Out of the total

number of live leukemia cells, 56.2% were in G_0-G_1 stage; 36.1% were in S stage; and 7.7% were in G_2-M stage, *i.e.*, 56.2% + 36.1% + 7.7% = 100% (total live leukemia cells).

In contrast, after culturing the HL-60 leukemia cells with untreated yeast cells for 72 hours (group NY), 11.6% of the leukemia cells were dead and 88.4% of the leukemia cells were alive (100% - 11.6%). Out of the total number of live leukemia cells, 41.6% were in G_0-G_1 stage; 40.2% were in S stage; and 18.2% were in G_2-M stage, *i.e.*, 41.6% + 40.2% + 18.2% = 100% (total live leukemia cells).

Similarly, after culturing the HL-60 leukemia cells alone for 72 hours (group CK), only 11.3% of the leukemia cells were dead and 88.7% of the leukemia cells were alive (100% - 11.3%). Out of the total number of live leukemia cells, 41.3% were in G_0-G_1 stage; 40.7% were in S stage; and 18.0% were in G_2-M stage, *i.e.*, 41.3% + 40.7% + 18.0% = 100.0% (total live leukemia cells).

After 72 hours, the percentage of programmed cell death is still significantly greater (8x) when the HL-60 leukemia cells were co-incubated with the activated and conditioned yeast cells of the present invention (85.2% in group AY) than when the HL-60 leukemia cells were co-incubated with the untreated yeast cells (11.6% in group NY) or cultured alone (11.3% in group CK). The percentage of programmed cell deaths in group AY increased more than three-folds from 24.7% to 85.2%, suggesting that the activated and conditioned yeast cells of the present invention are extremely effective in reducing the number of live leukemia cells. Further, more than half of the live leukemia cells in group AY at 72 hours (56.2%) are now in G_0-G_1 stage, *i.e.*, a temporary or permanent resting period where the cell has reached an end stage of development and will no longer divide.

The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

What is claimed is:

1. A biological composition comprising activated yeast cells, wherein said yeast cells are prepared by a method comprising at least two steps selected from the group consisting of:

(a) culturing yeast cells in a first electromagnetic field having a frequency in the range of 7,821 to 10,170 MHz and a field strength in the range of 200 to 350 mV/cm;

(b) culturing the yeast cells in a second electromagnetic field having a frequency in the range of 7,993 to 11,530 MHz and a field strength in the range of 190 to 330 mV/cm;

(c) culturing the yeast cells in a third electromagnetic field electromagnetic fields having a frequency in the range of 9,907 to 12,285 MHz and a field strength in the range of 230 to 430 mV/cm;

(d) culturing the yeast cells in a fourth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 220 to 450 mV/cm; and

(e) culturing the yeast cells in a fifth electromagnetic field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm.

2. A biological composition comprising activated and conditioned yeast cells, wherein the yeast cells are prepared by a method comprising activating the yeast cells, said activating comprising at least two steps selected from the group consisting of:

(a) culturing yeast cells in a first electromagnetic field having a frequency in the range of 7,821 to 10,170 MHz and a field strength in the range of 200 to 350 mV/cm;

(b) culturing the yeast cells in a second electromagnetic field having a frequency in the range of 7,993 to 11,530 MHz and a field strength in the range of 190 to 330 mV/cm;

(c) culturing the yeast cells in a third electromagnetic field electromagnetic fields having a frequency in the range of

9,907 to 12,285 MHz and a field strength in the range of 230 to 430 mV/cm;

(d) culturing the yeast cells in a fourth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 220 to 450 mV/cm; and

(e) culturing the yeast cells in a fifth electromagnetic field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm,

and conditioning the activated yeast cells, said conditioning comprising at least one step selected from the group consisting of:

(f) culturing the yeast cells in a liquid medium comprising wild hawthorn juice and gastric juice of a mammal in a sixth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 220 to 450 mV/cm; and

(g) culturing the yeast cells in a liquid medium comprising wild hawthorn juice and gastric juice of a mammal in a seventh electromagnetic field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm.

3. A biological composition comprising activated and conditioned yeast cells, wherein the activated and conditioned yeast cells of claim 2 are subjected to at least one period of culturing in a liquid medium comprising wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice, and in the presence of in any order:

(h) an eighth electromagnetic field or series of electromagnetic fields having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 170 to 450 mV/cm; and

(i) a ninth electromagnetic field or series of electromagnetic fields having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 120 to 450 mV/cm.

4. The biological composition of claim 1, 2, or 3, wherein the activated yeast cells are at a concentration of about 10^6 to 10^9 cells per ml.

5. The biological composition of claim 1, 2, or 3, wherein the activated yeast cells are dried and at a concentration of about 10^7 to 10^{10} cells per gram.

6. A pharmaceutical composition comprising the activated and conditioned yeast cells of claim 1, 2 or 3, and a pharmaceutical acceptable carrier.

7. A dietary supplement comprising the activated and conditioned yeast cells of claim 1, 2 or 3, and one or more ingredients selected from the group consisting of vitamins, herbs, herbal extracts, minerals, amino acids, metal chelates, plant extracts, coloring agents, flavor enhancers and preservatives.

8. A nutritional composition comprising the activated and conditioned yeast cells of claim 1, 2 or 3, and a food product selected from the group consisting of a fruit juice-based beverage, a tea-based beverage, a dairy product, a soybean product, and a rice product.

9. A method for preparing a biological composition comprising activated yeast cells, said method comprising at least two steps selected from the group consisting of:

(a) culturing yeast cells in a first electromagnetic field having a frequency in the range of 7,821 to 10,170 MHz and a field strength in the range of 200 to 350 mV/cm;

(b) culturing the yeast cells in a second electromagnetic field having a frequency in the range of 7,993 to 11,530 MHz and a field strength in the range of 190 to 330 mV/cm;

(c) culturing the yeast cells in a third electromagnetic field electromagnetic fields having a frequency in the range of 9,907 to 12,285 MHz and a field strength in the range of 230 to 430 mV/cm;

(d) culturing the yeast cells in a fourth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 220 to 450 mV/cm; and

(e) culturing the yeast cells in a fifth electromagnetic field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm.

10. A method for preparing a biological composition comprising activated and conditioned yeast cells, said method comprising activating the yeast cells, said activating comprising at least two steps selected from the group consisting of:

(a) culturing yeast cells in a first electromagnetic field having a frequency in the range of 7,821 to 10,170 MHz and a field strength in the range of 200 to 350 mV/cm;

(b) culturing the yeast cells in a second electromagnetic field having a frequency in the range of 7,993 to 11,530 MHz and a field strength in the range of 190 to 330 mV/cm;

(c) culturing the yeast cells in a third electromagnetic field electromagnetic fields having a frequency in the range of 9,907 to 12,285 MHz and a field strength in the range of 230 to 430 mV/cm;

(d) culturing the yeast cells in a fourth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 220 to 450 mV/cm; and

(e) culturing the yeast cells in a fifth electromagnetic field having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm,

and conditioning the activated yeast cells, said conditioning comprising at least one step selected from the group consisting of:

(f) culturing the yeast cells in a liquid medium comprising wild hawthorn juice and gastric juice of a mammal in a sixth electromagnetic field having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 230 to 440 mV/cm; and

(g) culturing the yeast cells in a liquid medium comprising wild hawthorn juice and gastric juice of a mammal in a seventh electromagnetic field having a frequency in the range of

12,031 to 12,900 MHz and a field strength in the range of 260 to 450 mV/cm.

11. A method of making a biological composition comprising activated and conditioned yeast cells, said method comprising culturing the activated and conditioned yeast cells prepared by the method of claim 18 in a liquid medium comprising wild hawthorn juice, jujube juice, wu wei zi juice, and soybean juice, and in the presence of in any order:

(h) an eighth electromagnetic field or series of electromagnetic fields having a frequency in the range of 11,141 to 12,842 MHz and a field strength in the range of 170 to 450 mV/cm; and

(i) a ninth electromagnetic field or series of electromagnetic fields having a frequency in the range of 12,031 to 12,900 MHz and a field strength in the range of 120 to 450 mV/cm.

12. The method of claim 10 or 11 further comprising after the culturing step drying the activated and conditioned yeast cells.

13. The method of claim 12, wherein the drying step comprises:

(a) drying at a temperature not exceeding 65°C for a period of time such that the yeast cells become dormant; and

(b) drying at a temperature not exceeding 70°C for a period of time to reduce the moisture content to below 5%.

14. A method for retarding the growth of cancer cells in a mammal comprising administering orally to the mammal an effective amount of the biological composition of claim 1, 2 or 3.

15. A method for prolonging the time of survival of a mammal with cancer comprising administering orally to the mammal an effective amount of the biological composition of claim 1, 2 or 3.

16. The biological composition of claim 1, 2 or 3 for use as a medicament.

17. Use of the biological composition of claim 1, 2 or 3 for the manufacture of a medicament for the treatment of cancer.

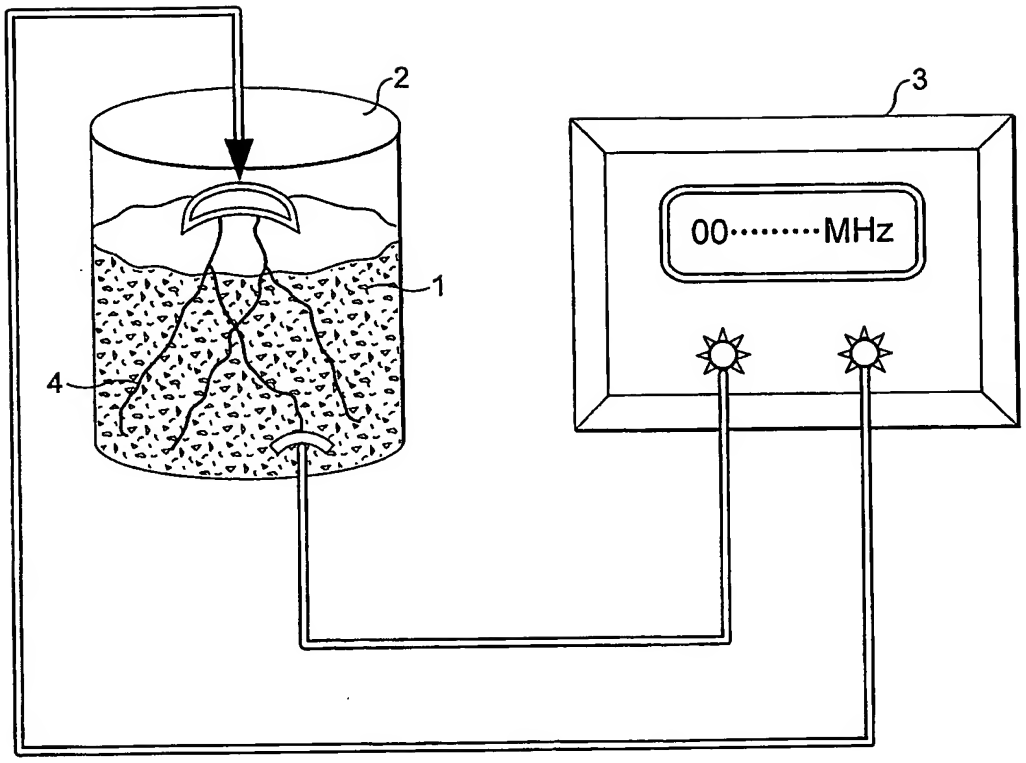

FIG. 1

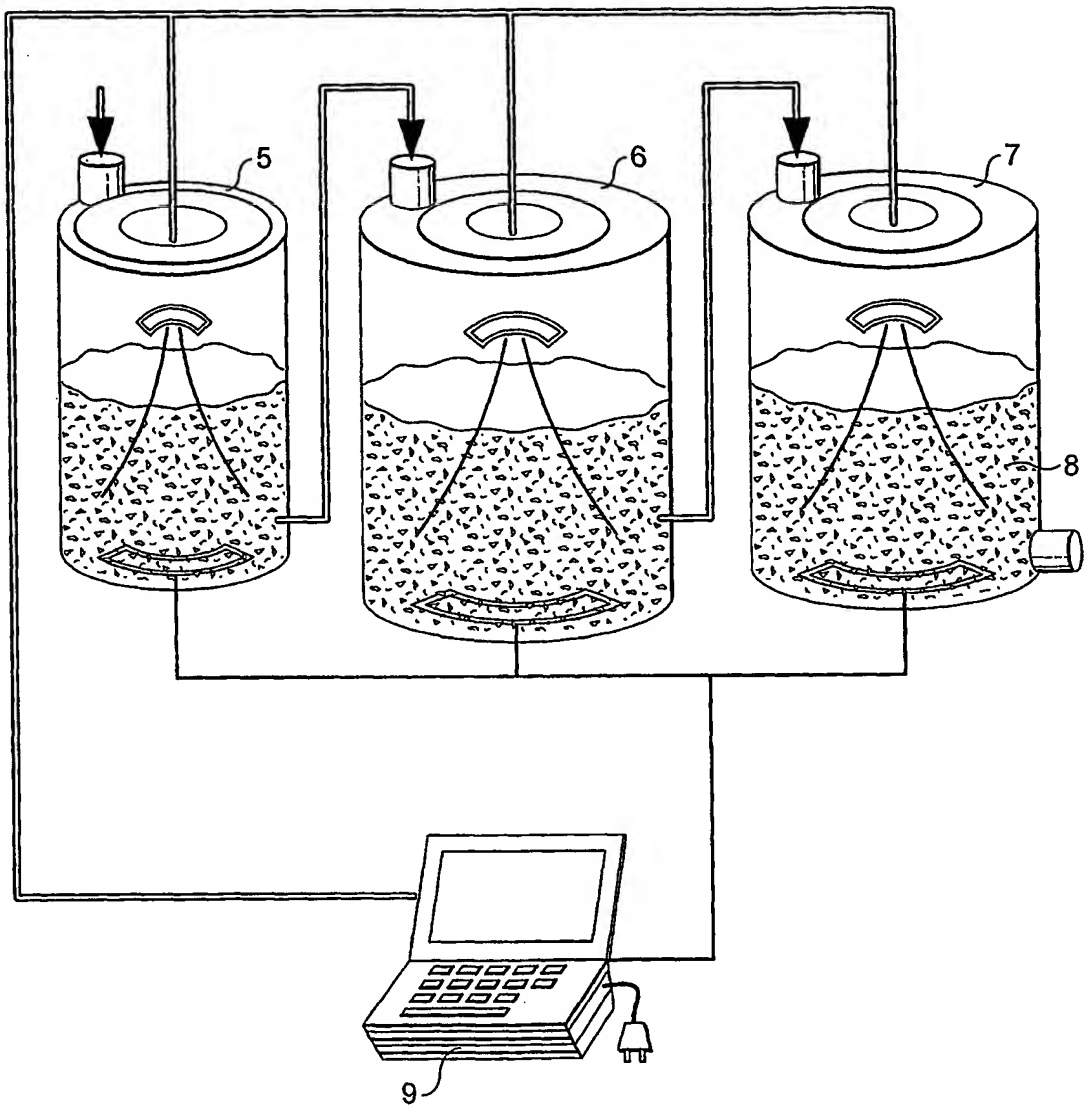

FIG. 2

INTERNATIONAL SEARCH REPORT

Internatio .plication No
PCT/GB2004/002466

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N13/00 C12N1/16 C12N1/18 A61K35/72 A61K41/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, MEDLINE, BIOSIS, EMBASE, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 200267 Derwent Publications Ltd., London, GB; Class B04, AN 2002-627548 XP002259470 & WO 02/062983 A1 (SIX FOREST BIO-SCI INST LTD) 15 August 2002 (2002-08-15)	1-8, 14-17
A	abstract	9-13
X	WO 02/070683 A (CHEUNG LING YUK ; ULTRA BIOTECH LTD (GB)) 12 September 2002 (2002-09-12)	1-8
A	claims 14-19	9-17

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 August 2004	25/08/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	Grötzinger, T

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2004/002466

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PONNE C T ET AL: "Interaction of electromagnetic energy with biological material--relation to food processing" RADIATION PHYSICS AND CHEMISTRY, ELSEVIER SCIENCE PUBLISHERS BV., AMSTERDAM, NL, vol. 45, no. 4, 1 April 1995 (1995-04-01), pages 591-607, XP004051598 ISSN: 0969-806X page 597; table 2 page 598 - page 599; table 3	1-8
A	page 602; table 4	9-17
X	BINNINGER D M ET AL: "Effects of 60Hz AC magnetic fields on gene expression following exposure over multiple cell generations using Saccharomyces cerevisiae" BIOELECTROCHEMISTRY AND BIOENERGETICS, XX, XX, vol. 43, no. 1, 1997, pages 83-89, XP002223047 ISSN: 0302-4598	1-8
A	page 83, right-hand column, line 13 - line 15 page 84, left-hand column, line 1 - line 4	9-17
P,X	EP 1 375 652 A (ULTRA BIOTECH LTD) 2 January 2004 (2004-01-02)	1-8,16
P,A	claims 1-30	9-15,17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2004/002466

Box II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 14 and 15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/002466

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 02070683 A	12-09-2002	US 6391617 B1	21-05-2002
		US 2002123127 A1	05-09-2002
		US 2002123130 A1	05-09-2002
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		EP 1375652 A2	02-01-2004